# Recovery-Related Brain Alterations after Mild Traumatic Brain Injury: A Longitudinal, Multimodal Imaging Approach

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## Summary

Mild traumatic brain injury (mTBI) is one of the most challenging neurological injuries and represents a public health issue worldwide. Although mTBI can cause various, sometimes persistent, symptoms, structural lesions cannot be detected with standard clinical magnetic resonance imaging (MRI) scans. To date, the long-term evolution of mTBI remains poorly defined, and no objective markers of recovery exist. The main objective of this thesis was to investigate early post-injury functional and structural brain alterations in mTBI patients compared with well-matched healthy controls, to monitor these neural alterations when patients transit from the acute to the chronic stage (≤ 7 days and 1-year post-injury, respectively) and to associate neural alterations with alterations in symptom severity and cognitive performance.

*Study I* explored connectivity alterations by means of resting-state functional and diffusion tensor MRI. In the acute phase, reduced functional connectivity was found in a network that overlaps the nodes of the default mode network. Moreover, a network of enhanced structural connectivity was identified that included central hubs such as the anterior and posterior cingulate cortex. These altered networks demonstrated strong inverse relations and anatomical similarity. Both networks recovered partially over the course of a year, and these recoveries were accompanied by cognitive improvements.

*Study II* evaluated morphological reorganization with the use of 3D T1-weighted MRI. The results showed an increase in cortical thickness that was spatially focused on prefrontal clusters and was not yet normalized in the chronic phase. Slight cortical thickening was associated with cognitive recovery in the good-outcome subgroup, while strong thickening was linked to cognitive decline in the poor-outcome subgroup, potentially indicating neuroinflammation.

Taken together, the results show that mTBI-induced neuroplasticity differs in time course between brain regions, with highly interconnected hubs being the slowest to recover. Residual neural alterations at 1-year post-injury emphasize the importance of monitoring the consequences of mTBI over a sufficiently long period. These findings have important clinical implications for prevention, intervention, and prognosis of mTBI.

# Zusammenfassung

Die leichte traumatische Hirnverletzung (LTHV) ist eine der herausforderndsten neurologischen Verletzungen und stellt weltweit ein öffentliches Gesundheitsproblem dar. Obwohl die Diagnose LTHV zu verschiedenen, manchmal langanhaltenden klinischen Symptomen führen kann, können mit konventionellen bildgebenden Verfahren keine strukturellen Hirnverletzungen nachgewiesen werden. Der langfristige Verlauf einer LTHV bleibt bis heute unzureichend geklärt; objektive neurobiologische Erholungsmarker existieren kaum. Das Hauptziel der vorliegenden Doktorarbeit war die Untersuchung von posttraumatischen funktionellen und strukturellen Hirnveränderungen bei Patienten mit LTHV in der Frühphase im Vergleich zu den entsprechenden gesunden Kontrollprobanden. Zudem wurde die Interaktion zwischen Gruppe und Zeit von verschiedenen neuronalen Messwerten von der akuten zur chronischen Phase (≤ 7 Tage und 1 Jahr nach dem Trauma) analysiert. Die Ergebnisse dieser Interaktion wurden dann in Verbindung zu den kognitiven Veränderungen gesetzt. *Studie I* erforschte Netzwerkveränderungen mittels funktioneller MRT im Ruhezustand und Diffusions-Tensor-Bildgebung. In der Frühphase wurde eine reduzierte funktionelle Konnektivität bei den Patienten in einem Netzwerk gefunden, dessen Knotenpunkte eine Überlappung mit Regionen des Default-Mode-Netzwerks aufwiesen. Zudem wurde ein Netzwerk mit erhöhter struktureller Konnektivität identifiziert, welches mehrere übergeordnete Kernknotenpunkte des Gehirns (sog. Hubs) umfasste. Diese beiden beeinträchtigten Netzwerke waren signifikant negativ korreliert und zeigten erhebliche anatomische Überlappungen. Beide Netzwerke erholten sich nur teilweise, und diese partielle Erholung ging mit kognitiven Leistungsverbesserungen einher. *Studie II* lieferte Einblicke in die morphologische Reorganisation mittels Verwendung der, T1-gewichteten MRT-Sequenz. Die Ergebnisse zeigten eine Zunahme der kortikalen Dicke, hauptsächlich in präfrontalen Strukturen, welche sich in der chronischen Phase noch nicht normalisiert hatte. Eine leichte kortikale Verdickung war mit einer kognitiven Erholung in der Subgruppe von Patienten mit gutem Verlauf assoziiert, während eine ausgeprägte kortikale Verdickung mit einer Verschlechterung der kognitiven Leistungsfähigkeit in der Subgruppe mit schlechtem Verlauf verknüpft war. Zusammengefasst zeigen die Ergebnisse dieser multimodalen Studie, dass die LTHV-bedingte Neuroplastizität sich im Zeitverlauf je nach Hirnregionen unterscheidet, wobei hochvernetzte Hubs sich am langsamsten zu erholen scheinen. Residuale neurale Veränderungen bzw. Kompensationsmechanismen ein Jahr nach einer Hirnverletzung betonen, dass es wichtig ist, die Auswirkungen einer LTHV über einen ausreichend langen Zeitraum zu verfolgen.

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# List of abbreviations

| | |
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| ACC | anterior cingulate cortex |
| APFC | anterior prefrontal cortex |
| CT | computed tomography |
| DAI | diffuse axonal injury |
| DLPFC | dorsolateral prefrontal cortex |
| DMN | default mode network |
| DTI | diffusion tensor imaging |
| FA | fractional anisotropy |
| FDR | false discovery rate |
| GCS | Glasgow coma scale |
| GO | good outcome |
| MPFC | medial prefrontal cortex |
| MRI | magnetic resonance imaging |
| mTBI | mild traumatic brain injury |
| NBS | network-based statistic |
| OFC | orbitofrontal cortex |
| PCC | posterior cingulate cortex |
| PCD | post-concussion disorder |
| PO | poor outcome |
| RPQ | Rivermead Post Concussion Symptoms Questionnaire |
| rsfMRI | resting-state functional magnetic resonance imaging |
| rTMS | repetitive Transcranial Magnetic Stimulation |
| SMA | supplementary motor area |
| STG | superior temporal gyrus |
| TBI | traumatic brain injury |
| tDCS | transcranial Direct Current Stimulation |
| TP | temporal pole |
| VLPFC | ventrolateral prefrontal cortex |

# 1 Theoretical background

## 1.1 Introduction

Traumatic brain injury (TBI) is among the most frequent neurological disorders and a major cause of disability. The Glasgow coma scale (GCS) primarily classifies cases of TBI as mild, moderate, or severe, with mild TBI (mTBI) being by far the most frequent form of TBI. In fact, mTBI constitutes about 80–90% of all brain traumas, with an estimated annual incidence of 100–300 cases treated at hospital per 100,000 people (Cassidy et al., 2004). To date, no precise data are available on the incidence of mTBI in the general population. Approximately 1.4–3.8 million cases of mTBI are predicted to occur every year in the USA (Laker, 2011; Coronado et al., 2015). But this rate is probably the tip of the iceberg, since it is likely that the majority of cases go unreported. A large number of patients are actually not treated in emergency rooms, especially among athletes and the military, and therefore an accurate estimate of mTBI is likely to be above 600/100,000 people (Cassidy et al., 2004). By comparison, the annual incidence of individuals experiencing mTBI in Switzerland amounts to 10,000 of all insured[1] under the federal law for accident insurance, according to the Central Office of Statistics. Men face approximately twice the risk that women do, and the condition affects young adults and teenagers disproportionately (Cassidy et al., 2004). Commonly known as concussion, mTBI frequently occurs in a wide variety of activities, including falls, sports injuries, road traffic accidents (motor vehicle, motorcycle, and bicycle collisions), assaults, military training, and combat-related events such as blast exposure. Functional outcome after mTBI is widely heterogeneous, but its high prevalence and incidence mean that the resulting economic and social burdens are considerable (Levin and Diaz-Arrastia, 2015).

Mild TBI leads to acute alterations in neurological, cognitive, somatic, motor, and emotional functioning that resolve within the first few days to weeks post-injury in most patients. Indeed, the vast majority of patients exhibit both spontaneous resolution of clinical symptoms and recovery by traditional neurobehavioral measures within 12 weeks (Bigler, 2008; McCrea et al., 2013). Consequently, mTBI has been considered as a minor and fully reversible injury for a long time.

However, a significant minority of mTBI patients experience long-term consequences severe enough to impair their quality of life and to interfere with occupational and social functioning. For this “miserable minority,” the residual symptoms or deficits may protract and persist up to years (Sterr et al., 2006; Zumstein et al., 2011; McMahon et al., 2013). Poor outcome after

[1] https://www.unfallstatistik.ch/d/publik/unfstat/unfstat_d.htm; SSUV-UVG Pool 2006-2010

mTBI, also called “postconcussion syndrome” or “postconcussion disorder”, can cause disability, loss of productivity, and high costs, with enormous economic impact on the public health system (Coronado et al., 2013).

Despite these observations, standard clinical imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are typically normal, and so incapable of providing prognostic information about the extent of the damage and the course of the disorder. This characteristic provided support for the idea that mTBI does not damage the macroscopic structures. Currently, widely cited reports of a link between repetitive mTBI and increased risk for the development of neurodegenerative diseases have attracted much attention to the diagnosis of mTBI. Repeated exposure to head impacts has long been suspected of producing cumulative, deleterious effects. In the recent past, the dramatic increase in diagnoses of chronic traumatic encephalopathy (CTE) amongst deceased athletes and military veterans suggests that life-long effects of mTBI may be more severe than initially believed (McKee et al., 2013). CTE, which has replaced the term “dementia pugilistica”, is a progressive neurodegenerative disorder characterized by tau pathology and brain atrophy (McKee et al., 2009). This tauopathy is suspected to cause cognitive and behavioral disturbances. The same concern applies to the early onset of Alzheimer’s dementia and increased depression observed in retired contact sports athletes (Hart et al., 2013; Lee et al., 2013; Strain et al., 2013; Gardner et al., 2014). Finally, a new body of evidence shows that even multiple subconcussive blows to the head without the diagnosis of mTBI can result in brain changes and poorer neurocognitive performance (Lipton et al., 2013; Bahrami et al., 2016).

Clinicians and researchers are required to revise their understanding of mTBI as a completely reversible event that can be replicated without risk. Partially due to the recent media spotlight on its dangers, especially in the USA, public awareness of mTBI has experienced a surge.

Despite the absence of evidence of structural lesions on conventional CT and MRI scans, novel neuroimaging advances have led to a proliferation of studies using different MRI modalities. These works have produced new insights into functional and structural anomalies in the acute, sub-acute, and chronic phases of mTBI. Nevertheless, accurate longitudinal investigations that follow the dynamic evolution of brain injury are still very limited.

The rest of this chapter discusses the background to the present thesis. Chapter 1 also includes an overview of the neuroimaging modalities applied, namely (1) resting-state functional MRI, (2) diffusion tensor MRI, and (3) high-resolution T1-weighted MRI. Chapter 2 outlines the aims and the main research questions, highlighting the relevance of this thesis. Chapter 3

describes the study sample and the longitudinal design. Chapter 4 presents two empirical works (Study I and II), which constitute the quintessence of this thesis. Finally, Chapter 5 comprises a general discussion and provides suggestions for clinical management and future research in this field.

## 1.2 Definition and classification of mTBI

The American Congress of Rehabilitation Medicine (ACRM)[2] defines mTBI as a traumatically induced physiological disruption of brain function resulting from the head being struck or striking an object or the brain undergoing an acceleration/deceleration movement without direct external trauma to the head, as manifested by at least one of the following:

(1) any period of loss of consciousness (LOC) up to 30 minutes

(2) any alteration in mental state at the time of the accident (e.g., confusion or disorientation)

(3) any loss of memory immediately before or after the event with a post-traumatic amnesia (PTA) not exceeding 24 hours

(4) GCS score of 13–15 after 30 minutes post-injury

(5) focal neurological deficit(s) that may or may not be transient

(6) CT, MRI, and other routine neurological evaluations may be normal.

Of note, *acceleration movement* means the head is hit by an object, *deceleration* means the head hits an object (Martin, 2016).

In 1999, a European Task Force on mTBI was set up with the support of the European Federation of Neurological Societies (EFNS). They proposed a definition of mTBI in alignment with the international guidelines (Vos et al., 2002; Vos et al., 2012).

The operational criteria for clinical identification include:

- GCS: 13–15 at hospital admission
- LOC: 30 minutes or less if present
- PTA: 60 minutes or less if present
- Retrograde amnesia (memory loss for the period before the accident): 30 minutes or less if present

The mTBI patients recruited for this PhD thesis were selected according to EFNS criteria.

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[2] American Congress of Rehabilitation Medicine (1993). Definition of mild traumatic brain injury. *J Head Trauma Rehabil* 8**,** 86-88.

One challenge facing the field is the lack of unanimity regarding the definition of mTBI for diagnosis, as different agencies report different criteria. A review performed by the WHO Task Force of 313 studies found 38 different definitions that, beside common overlapping criteria, also illustrated substantial differences (Carroll et al., 2004a). While the majority (62%) considered the GCS score as the sole component, others (38%) considered only LOC or amnesia.

Similar to the ACRM guidelines, some criteria also specify mTBI as an injury that does not result in any visible pathology that can be observed on common neuroradiological scans, while other authors have proposed the nomenclature "complicated mTBI" to include intracranial lesions or skull fractures detected by CT on the day of injury (Wrightson and Gronwall, 1981; Williams et al., 1990). In any case, intracranial complications after mTBI are infrequent (10%) and rarely require neurosurgical intervention (Vos et al., 2012).

The term mTBI is further encumbered by a range of synonyms, such as mild or minor head injury, mild closed head injury, commotio cerebri, and concussion, all of which can be found in the literature. Some research groups restrict the term concussion to the mildest end of mTBI or use it exclusively in sporting contexts.

Continued efforts to reach global consensus on a common terminology and diagnostic coding of mTBI are essential.

## 1.3 Persistent post-concussion disorder

Post-concussion symptoms include *physical* symptoms (e.g., headache, dizziness, nausea, sleep disturbance, fatigue, restlessness, blurred vision, sensitivity to light and noise), *cognitive* symptoms (e.g., problems with memory, attention/concentration, and executive functions, slowed thinking), as well as *behavioral* or *emotional* symptoms (e.g., emotional lability, sadness/depression, irritability, anxiety, frustration). These symptoms usually resolve within 3 months after injury.

Patients who have post-concussion complaints for longer time periods are referred to as developing a post-concussion syndrome or post-concussion disorder (PCD). PCD is a complex disorder for which no clear guidelines exist, rendering it difficult to predict who is likely to develop a PCD. The diagnosis and nature of the disorder have been the subject of strident debate since the late 19th century (Benton et al., 1989).

Currently, there are two sets of research criteria for the diagnosis of PCD: those published in the *10th International Classification of Diseases ICD-10* (World Health Organization, 1992), and those appearing in the 4th edition of the *Diagnostic*

*and Statistical Manual of Mental Disorders* DSM-IV (American Psychiatric Association, 1994).

- According to the ICD-10 criteria, a person must have a history of head trauma preceding the onset of symptoms by a period of up to 4 weeks and symptoms in at least three of six categories.
- According to the more stringent DSM-IV criteria, a person must have a history of head trauma, report at least three of eight symptoms, which must be present for at least 3 months, and show objective evidence of neurocognitive deficits and significant impairment in social or occupational functioning.

Both formulations have been largely criticized, in part because they do not report lower and upper limits of severity, and the symptoms listed for these two definitions are not specific to mTBI (Carroll et al., 2004a). In practice, clinicians frequently rely on checklists or questionnaires to document post-concussion symptoms. For example, the popular Rivermead Post Concussion Symptoms Questionnaire (RPQ) has demonstrated both validity and reliability (King et al., 1995; Crawford et al., 1996). This questionnaire compares not only the presence but also the *severity* of 16 symptoms after mTBI with pre-injury levels.

Despite decades of research, the diagnosis of PCD as a true disorder remains controversial and the criteria for defining PCD need to be reviewed. Without question, acute PCD can be caused by the pathophysiology of mTBI, but concern is related to the specificity of *chronic* PCD. Does this have an entirely neurobiological basis?

In this context, it is important to recognize that mTBI is not the only cause of such non-specific constellations of symptoms and problems. The general population also reports similar complaints, and one mTBI study found that 31% of their healthy control sample met the ICD-10 symptom criteria for PCD (Waljas et al., 2015). In addition, these non-specific deficits rely on self-reports and subjectivity, so reporting bias may be introduced whenever external incentives may be involved. For example, the tremendous desire to return-to-play in athletes may result in the under-reporting of neurobehavioral symptoms after concussion; in contrast, the potential for financial compensation in accident-related litigation may lead to over-reporting of symptoms (Bianchini et al., 2005; Greenwald et al., 2012; Kristman et al., 2014). Accordingly, there is a lack of agreement on the prevalence of PCD after mTBI. Estimates vary considerably (24-84%), depending on diagnostic criteria, outcome measure, and follow-up interval (Ryan and Warden, 2003). At 1 year after injury, between 10% and 30% of mTBI patients continue to perceive symptoms and could diagnosed as PCD (Alexander, 1995; Sterr et al., 2006; McMahon et al., 2013).

The findings on persistent *cognitive* impairments after mTBI are also heterogeneous. A meta-analysis of neuropsychological outcome re-examined 25 studies with a total sample size of 2,834 mTBI patients and 2,057 control participants and detected complete recovery within 3 months of injury (Rohling et al., 2011). Conversely, a prospective study reported poorer performance on a visual memory task in a group of 123 mTBI patients compared to a group of controls at 3 months post-injury (Ponsford et al., 2011). This study found no group differences in psychiatric states, and 93% of mTBI participants were in employment at follow-up.

Chronic symptoms can be caused, maintained, or exacerbated by a multitude of vulnerabilities unrelated to the neurobiology of the damage, such as pre-existing conditions or comorbidities. These pre- or co-occurring factors include chronic pain, migraine, insomnia, substance dependence, depression, post-traumatic stress disorder and anxiety disorders, or an interaction of these factors (Radhakrishnan et al., 2016). Indeed, all these confounding problems have some symptoms that can mimic those of PCD and can lead to a misdiagnosis of the latter, helping to promote the belief that only mTBI patients with previous psychiatric histories are likely to remain chronically symptomatic. Nowadays, psychiatric diseases prior to mTBI are often exclusion criteria for research into long-term outcome. On the other hand, there is evidence that increased risk of psychiatric disorders is associated with mTBI (Teasdale and Engberg, 2001; Carroll et al., 2014). A phase II study among patients with no psychiatric diagnosis in the year before the injury found almost a threefold risk of a psychiatric illness in the first 6 months and an increased risk through the first 2 years post-mTBI (Fann et al., 2004).

Predictors of adverse outcomes in the aftermath of mTBI are not known with certainty, but certain pre-, peri-, and post-injury factors appear to increase the risk (see also Chapter 5.3). Beside pre-existing psychiatric disorders, female gender, previous concussions, low academic or socioeconomic status, litigation stress, as well as psychological and social characteristics (e.g. misattributions, expectations, psychological distress, coping strategies), are just a few of the variables thought to constitute pre-injury factors (Dischinger et al., 2009; Meares et al., 2011; Ponsford et al., 2011). Post-injury factors include the presence of extra-cranial bodily injuries and being symptomatic in the early phase post-injury (Waljas et al., 2015).

The etiology and the degree to which physiological and psychological effects of the brain trauma are responsible for persistent PCD are not known. Although the brain of a mTBI patient typically appears normally on conventional imaging sequences, novel neuroimaging techniques have shown subtle structural and functional brain anomalies associated with PCD (see Chapter 1.4). However, advanced MRI modalities have also yielded inconsistent findings. In both

studies of this thesis, we not only consider the mTBI sample as a single group, but also focus on the subgroup of patients with prolonged complaints.

## 1.4 Pathophysiology of mTBI

The understanding of the neurobiology of mTBI has expanded dramatically in the past decade, leading recently to a significant shift in thought regarding its consequences. Additionally, it is increasingly possible to speculate on links between physiological perturbations and clinical characteristics, especially in the early phase.

Traditionally, mTBI was understood as a purely *physiological* disruption in which biomechanical forces stretch the axons, leading to changes in ionic gradients, neurotransmitter imbalance, and neuroinflammation (Giza and Hovda, 2001). These events cause injury to the neural cell membranes. Briefly, the shear-strain effects of the trauma alter the balance of intracellular and extracellular ions by opening ion channels: calcium and sodium that normally remain outside the cell membrane enter, while potassium effluxes from the neuron (Bigler and Maxwell, 2012). This uncontrolled ion flux across the membrane, which is also thought to provoke migraine headaches, triggers the release of excitatory neurotransmitters such as glutamate (Giza and Hovda, 2014). Neurotransmitter alterations contribute to the acute clinical symptoms of mTBI, making thinking, moving, and talking much harder (Giza and Hovda, 2014). To restore the membrane deformity, the sodium-potassium pump must go into overdrive, transporting potassium into and sodium out of the cell. This process is energy-expensive and causes an increased demand for glucose (hyperglycolysis), dramatically depleting cellular stores of energy. The effort of the cell to restore the ionic homeostasis leads to an "energy crisis" (Giza & Hovda 2001).

The increased demand for energy in the injured brain provides explanations for certain clinical observations. One example is the increased vulnerability to repeat injury in the days following the initial injury, which is particularly crucial for the management of sports-related concussion (Guskiewicz et al., 2003; McCrea et al., 2009; McCrory et al., 2013). Clinically, it is accepted that a prior concussion is a risk factor for a second, with a peak around 10 days post-injury (Giza et al., 2013). Another characteristic of the energy crisis is symptom exacerbation when competing mental or physical demands occur, which also compromises the recovery process (Kerr et al., 2011; Silverberg and Iverson, 2013). Left alone, the energy crisis typically resolves within a relatively short time, days to weeks. Some experts suggest that this time frame is consistent with the neuropsychological recovery from mTBI (Belanger et al., 2005).

The neurometabolic and neurochemical cascade discussed above provide plausible explanations for certain clinical phenomena. However, other observations from clinical research and practice are not well explained by these pathophysiological processes. For instance, the protracted symptoms in a subgroup of patients and the emerging evidence of long-term pathological consequences of mTBI are difficult to explain with the transient neurometabolic cascade.

More recent research supports a mechanism for persistent neural injury in which *axon damage* is thought to be the predominant pathological mechanism underlying mTBI (Inglese et al., 2005; Niogi et al., 2008; Mayer et al., 2010a). Long association fasciculi such as the corpus callosum, the cingulum, the inferior frontooccipital fasciculus, and the longitudinal fasciculus are considered particular vulnerable to shear-strain deformations resulting from accelerational, decelerational, or rotational forces (Bendlin et al., 2008; McAllister and Stein, 2010; Chatelin et al., 2011; Bigler and Maxwell, 2012). Mounting evidence suggests that biomechanical forces can damage the delicate microstructural components of axons, including neurofilaments and microtubules, leading to *diffuse axonal injury* (DAI). Although DAI is best described in moderate-severe TBI as disruption of long white matter tracts, a growing body of human and animal research supports an axonal pathology in mTBI too (Mac Donald et al., 2007; Bigler and Bazarian, 2010; Spain et al., 2010; Browne et al., 2011; Bigler, 2013). Furthermore, microscopic DAI were found in a histopathological study of some mTBI cases that died from causes other than mTBI (Bigler, 2004). Advanced neuroimaging techniques, in particular diffusion tensor imaging (DTI), have brought about a step change in the ability to identify and quantify DAI. DTI enables researchers to estimate the orientation of axonal fibers in vivo, based on the fact that water diffuses most rapidly along the length of axons. Fractional anisotropy (FA), the most commonly used DTI parameter, measures the directionality of water diffusion and therefore the degree of structural integrity of white matter tracts (Stieltjes et al., 2001; Mori and Zhang, 2006). Higher FA indicates more water diffusion in a particular direction. In mTBI, water diffusion is restricted due to axonal swelling or cytotoxic edema, and higher FA than in controls may indicate an inflammatory response (Mayer et al., 2010a; Bigler, 2013). On the other hand, decreased FA in mTBI indicates less directionality and may reflect axonal degradation and damage to myelin or axon membrane. Here, intracellular and extracellular water mix, because the axon membranes do not effectively constrain water or are now absent (Bigler, 2013). According to reviews, changes in FA may reflect microstructural changes at different stages post-injury, with a tendency to increased FA in the acute phase and decreased FA in the chronic phase (Niogi and Mukherjee, 2010; Eierud et al., 2014). The FA

index has also yielded correlations with acute and chronic cognitive impairments (Kraus et al., 2007; Niogi et al., 2008; FitzGerald and Crosson, 2011; Kinnunen et al., 2011).

DTI anomalies have also been associated with an increase in severity of PCD symptoms, suggesting that greater damage to axonal integrity causes more severe PCD symptoms (Bazarian et al., 2007; Niogi et al., 2008; Wilde et al., 2008; Messe et al., 2011; Smits et al., 2011; Messe et al., 2012). However, such anomalies have been reported in a number of diverse brain regions, and other studies have not found any correlation (Lange et al., 2012; Ling et al., 2012; Ling et al., 2013; Waljas et al., 2013; Waljas et al., 2015).

Other advanced MRI techniques, such as functional MRI (fMRI), positron emission tomography (PET), and magnetic resonance spectroscopy, have detected some changes associated with adverse outcome (Chen et al., 2007; Kirov et al., 2013). However, findings are also heterogeneous here. No consensus has emerged in the fMRI literature; while certain authors observed PCD-specific alterations in functional brain networks (Stevens et al., 2012; Messe et al., 2013), others found no significant neuronal activation differences after multiple sports-related concussions (Terry et al., 2012). PET studies have also shown conflicting results in the PCD population, with some reporting an association (Ruff et al., 1994; Otte et al., 1997) and others not (Chen et al., 2003).

Nevertheless, new evidence suggests that a history of repetitive traumatic impact may trigger deleterious long-term cognitive, emotional, and behavioral alterations, leading to progressive neurodegenerative processes related to CTE and Alzheimer's disease (Johnson et al., 2010; McKee et al., 2013; Gardner et al., 2014; Koerte et al., 2015; Strain et al., 2015).

## 1.5 Multimodal MRI of the brain

Combining neuroimaging data using different modalities and techniques has begun to be popular and is growing exponentially in neuroscience research. In this thesis, we define multimodal neuroimaging as the combination of information from distinct MRI sequences. Multimodal neuroimaging has also become one of the major drivers in achieving higher accuracy than the best individual metric and thus overcoming the limitations of single modalities. In particular, multimodal MRI approaches have been increasingly applied in the detection, diagnosis, and prognosis of neurological and psychiatric disorders (Liu et al., 2015a). To capture the multi-faceted pathology of mTBI, studies that integrate multiple imaging modalities can concentrate on the same and related phenomena from different angles, and findings from different sources may be cross-validated.

Current neuroimaging techniques can be broadly classified into *functional* and *structural*

neuroimaging. Accordingly, the categories of multimodal approaches include a functional-structural combination, a structural–structural combination, or a functional–functional combination (Liu et al., 2015a).

In this thesis, we focus on functional-structural and structural-structural analysis by combining resting-state functional MRI (rsfMRI), diffusion MRI using DTI and structural high-resolution T1-weighted MRI recordings (Figure 1).

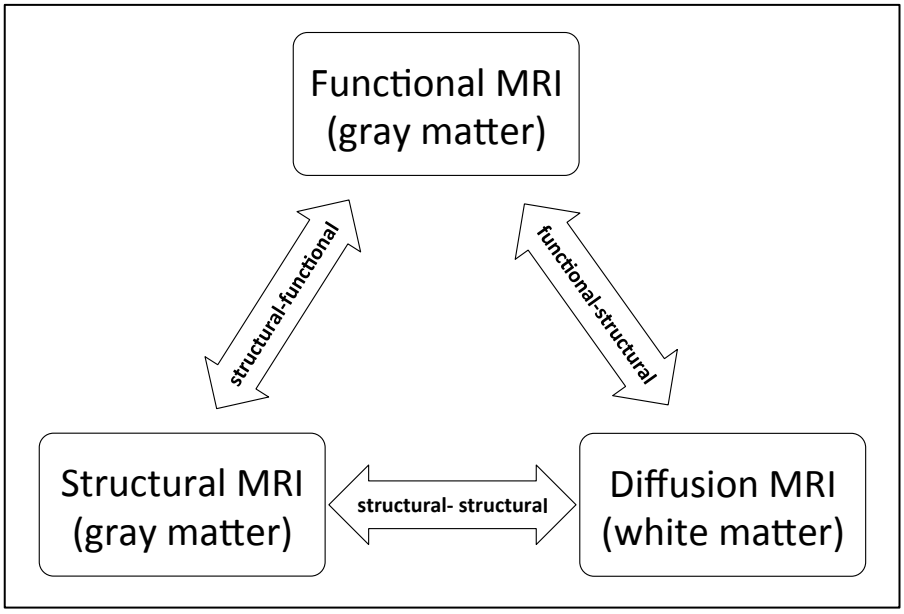


**Figure 1. Overview of the multimodal neuroimaging techniques used in the present thesis**

To date, the *functional-structural* combination is largely used because of its capacity to identify the relationship between brain function and structure. One powerful approach is the combination of functional and structural MRI sequences (rsfMRI & DTI) to gain insight into the *connectivity* of gray and white matter. Nowadays, the brain is commonly modeled as a network of functional and structural connections with the aim of establishing how changes in function are related to underlying changes in structure. Structural connections correspond to physical links, which form the anatomical framework for neuronal communication and signaling. Conversely, functional connections in the resting brain represent statistical dependencies (i.e. co-activations) between spatially distinct brain regions forming resting-state networks. It is increasingly recognized that structural connectivity is essential for brain activity. Authors exploring this association in healthy participants report that the strength of functional connectivity is positively correlated with structural connectivity (Damoiseaux and Greicius, 2009; Deco and Corbetta, 2011). However, studies examining the relationship

between functional and structural connectivity in mTBI are not yet available. When looking at moderate to severe TBI, two studies so far have compared functional and structural connectivity by means of graph-theoretical network analysis (Sharp et al., 2011; Caeyenberghs et al., 2013). One study found that greater white matter disruption was related to less functional connectivity within the default mode network (Sharp et al., 2011). The second study, however applying task-related fMRI, yielded very weak to zero correlations between graph metrics of functional and structural connectivity (Caeyenberghs et al., 2013).

Another common *functional-structural* combination is the exploration of gray matter properties within the same tissue class (gray matter) using rsfMRI and high-resolution T1-weighted images. Until now, only one study has jointly investigated task-evoked fMRI and cortical thickness measurements following mTBI (Urban et al., 2016). The authors reported a possible relationship between thinner cortices and hypoactivation in the left dorsolateral prefrontal region and in the right inferior parietal lobe.

Finally, the *structural-structural* category is dominated by diffusion and structural MRI (DTI & T1-weighted imaging). This combination provides information on white and gray matter morphometry and has already been used in mTBI studies (Toth et al., 2013; Narayana et al., 2015). Both longitudinal studies found a normalization of the axonal pathology (i.e. of mean diffusivity) in the first weeks, although the authors did not report any correlation with the results of the volumetric analysis.

Therefore, in the first study of this thesis (Study I), rsfMRI and DTI were combined to clarify changes of functional and structural connectivity in the acute and chronic phases of mTBI. The functional-structural relationship was also examined and discussed. In the second study (Study II), an additional third sequence, that is high-resolution T1-weighted MRI, was used to analyze changes in cortical gray matter morphology by considering the same time interval and the same sample.

# 2 Aims and research questions

By far the most prevalent neurological injury, mTBI has recently gained public attention as an important global health issue. Despite prevention campaigns all over the world, an end to accidents and injuries potentially leading to mTBI is not yet in sight. This challenging situation has stimulated researchers in the field to identify neuroimaging markers of the acute and chronic mTBI stage for the quantification of brain damage at micro- and macroscale levels.

The importance of incorporating longitudinal information to understand the dynamic nature of mTBI has been emphasized in many reviews (Mechtler et al., 2014; Xiong et al., 2014; Yuh et al., 2014; Pacifico et al., 2015; van der Horn et al., 2015a). Only studies with longitudinal design can help to track recovery-related trajectories and reorganization patterns, investigate the role of neuroplasticity, and predict the progression of brain injury over time. Findings from longitudinal studies have therefore clinical applications and can be used to revise management and intervention options. Despite the potential value of such observations, there is a scarcity of longitudinal studies in this area. The few existing studies, described in the introduction section of Study I and II (Chapter 4), have demonstrated divergent and partially contradictory results with regards to recovery evolution, spanning from zero to partial normalization. Thus, it is uncertain to date how the concussed brain recovers and how patterns of recovery differ from patterns of non-recovery. Not only are longitudinal studies using the single neuroimaging modality approach lacking, the absence of studies combining multimodal neuroimaging analyses is even more critical.

Furthermore, a crucial factor when performing MRI investigations of mTBI is the time passed since injury. To my knowledge, the majority of longitudinal studies have chosen to monitor patients' recovery to a maximum of 4-6 months after injury. However, a longer period may reveal more insights into the spectrum of long-term changes and may be necessary to reach firm conclusions on the typical time of recovery from mTBI (Mayer et al., 2011; Gama Sosa et al., 2014; Mouzon et al., 2014).

Another gap emerging from the mTBI literature appears in statistical analysis, since most empirical studies claiming to have adopted a longitudinal design did not take full advantage of the interaction between the variables of *time* (acute time point versus chronic time point) and *group* (patients versus healthy controls). Instead, they applied or report only within-group effects (i.e. effects in patients over time) and/or between-group effects (i.e. group differences at the beginning and end of the longitudinal period). In some studies, the healthy control group has only been scanned once, as the authors did not expect significant changes over time. Only the analysis of the group-by-time interaction is able to reduce confounding factors

unambiguously.

The *first overall aim* of this thesis was to characterize spontaneous injury-induced neuroplasticity in a typical sample of mTBI patients compared to changes in healthy control subjects over an extended interval of 1 year. Accordingly, Study I combined analysis of rsfMRI with a DTI sequence, while Study II addressed this purpose with the use of high-resolution T1-weighted MRI scans. Specifically, Study I focused at identifying alterations in patterns of functional and structural connectivity with the help of a graph-theoretical approach and at examining the relationship between the functional and structural network changes. Complementarily, Study II aimed at exploring the cortical reorganization of surface-based morphological measures — cortical thickness, surface area, and volume — from the acute to the chronic phase.

The *second overall aim* of this thesis was to broaden the current understanding of long-term associations between brain changes and cognitive changes within patients. This information could help to resolve whether the improvement in cognitive performance occurring in the majority of mTBI patients after the acute stage is actually attributable to recovery of the brain. So far, this relation has neither been examined with network analysis nor analyzed using surface-based morphometry. Therefore, Study I had the goal of linking alterations in cognitive abilities to alterations in functional and structural connectivity over the 1-year period, whereas Study II investigated this same relationship in cortical gray matter morphology.

Finally, understanding the pathophysiological mechanisms causing PCD is essential to identify instruments that reduce the quantity and the severity of deleterious mTBI-related effects. A specific marker for PCD would help to predict early which patients are more likely to be affected in the longer term and thus provide the basis for treatments that target anatomical anomalies. As reported in Chapter 1.3, a substantial minority of patients is at risk for experiencing protracted complaints that impact their quality of life. However, longitudinal studies, particularly important for detecting outcome-specific progressions, are scarce in this area too. A few studies targeting a measurement window of less than 6 months have reported axonal damage that partly recovered in patients without PCD but not in patients with PCD (Messe et al., 2011; Messe et al., 2012). Similar findings have been found in rsfMRI data, where the connectivity strength within the default mode network (DMN) was reduced in patients suffering from PCD compared to PCD-absent mTBI patients during both the acute and the chronic stages (Sours et al., 2015a). To this extend, the *third and final overall objective* of this thesis was to identify distinct outcome trajectories in patients with and without PCD in

order to differentiate the recovery-related dynamics behind good outcome (GO) and poor outcome (PO). Therefore, Study I monitored and compared the evolution of the two clinical subgroups with functional and structural connectivity parameters; Study II did the same with cortical morphometric parameters.

# 3 Methods

The present thesis focuses on data collected within the scope of the mTBI project conducted at Bellikon Rehabilitation Clinic in the canton of Aargau, Switzerland. This project is part of a range of measures taken by SUVA with the aim of improving understanding of mTBI and optimizing its acute and chronic management. It also comprises electrophysiological data investigated at three-time points during one year, but these data are outside the scope of this thesis.

## 3.1 Study sample

The recruitment of patients started in February 2012 and follow-up assessments continued until March 2015. The study was conducted in cooperation with the emergency departments of the University Hospital Zurich, the Aarau Cantonal Hospital, the Baden Cantonal Hospital, and the Waid Hospital Zurich. The enrolment and investigation of healthy control subjects occurred within the same period.

To qualify for participation, the patients had to meet the mTBI criteria recommended by the EFNS (Vos et al., 2002; Vos et al., 2012), had to be older than 18 but younger than 64 years, and had to show negative findings on CT scan. Mixed etiologies of sport- and nonsport-related brain injury were accepted. Each control subject was matched to the mTBI patient by gender, age, and years of education. To avoid possible bias due to confounding variables, most pre-existing conditions, such as history of psychiatric and neurological disorders, drug and alcohol abuse, and developmental cognitive disorders were carefully excluded for all participants. Further, only subjects who attended both visits and showed no intracranial anomalies on conventional MRI were admitted to the final analyses of this thesis. Finally, the data of 49 patients (mean age = 34.9 years; SD = 12.4 years) and 49 healthy controls (mean age = 35.0 years; SD = 12.1 years) were utilized for Studies I and II. Further details of the study sample are given in the empirical part (Chapter 4).

In accordance with the ICD-10 criteria, the RPQ was administered to identify PCD. Six patients reported three or more moderate to severe symptoms persisting for 1-year post-injury and were therefore assigned to the PO subgroup. The remaining 43 patients were assigned to the GO subgroup.

## 3.2 Longitudinal design

All participants were examined twice. Patients were investigated within the first 7 days after injury (Visit 1, designated as the acute phase) and after a 1-year interval (Visit 2,

designated as the chronic phase). Each visit consisted of two different parts, a MRI and a neuropsychological session (Figure 2). The first visit also included a neurological examination for the patients' group.

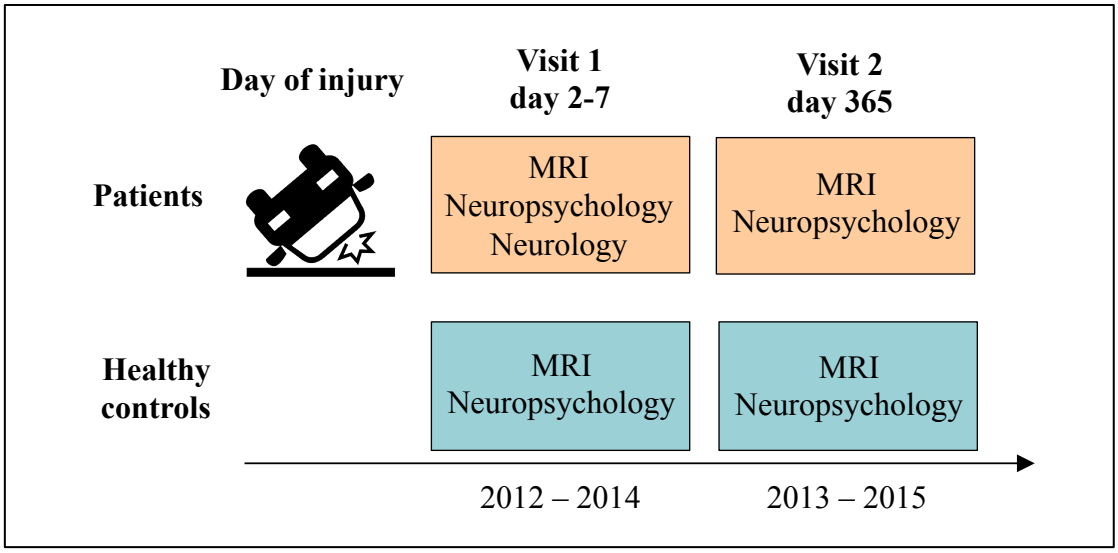


**Figure 2. MTBI study design**

The neuropsychological part took approximately 1.5 hours and comprised psychometric tests capturing diverse cognitive functions including measures of malingering and spatial intelligence as well as clinical surveys covering emotional, cognitive, and physical sequelae. Within the same day, or on different days within the same week, the participants underwent functional (resting-state T2*-weighted) and structural (T1-, T2- and diffusion-weighted) MRI examinations with a duration of about 1 hour. Control subjects were invited for data collection at equal times as the patients and completed the same assessments.

For both studies in this thesis, the neuroimaging investigations were performed at an average of 4.9 days (SD = 1.5 days) post-injury, and the interval between scans was 365.9 days (SD = 4.0 days) for the mTBI group and 364.6 days (SD = 5.1 days) for the control group.

Prospective observations of mTBI patients frequently yield a high rate of dropouts, leading to incomplete outcome data. A significant proportion of patients often decline to return for further testing or cannot be contacted again. Aware of this problem, considerable incentives were offered to maximize long-term motivation, such as financial compensation upon completion of the follow-up visit, intensive contact strategy (participants were contacted several weeks and some days before Visit 2), free transport service and free lunch.

# 4 EMPIRICAL PART

## 4.1 STUDY I

### Functional and structural network recovery after mild traumatic brain injury: a 1-year longitudinal study

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### 4.1.1 ABSTRACT

Brain connectivity after mild traumatic brain injury (mTBI) has not been investigated longitudinally with respect to both functional and structural networks together within the same patients, crucial to capture the multifaceted neuropathology of the injury and to comprehensively monitor the course of recovery and compensatory reorganizations at macro-level. We performed a prospective study with 49 mTBI patients at an average of 5 days and 1-year post-injury and 49 healthy controls. Neuropsychological assessments as well as resting-state functional and diffusion-weighted magnetic resonance imaging were obtained. Functional and structural connectome analyses were performed using network-based statistics. They included a cross-sectional group comparison and a longitudinal analysis with the factors group and time. The latter tracked the subnetworks altered at the early phase and, in addition, included a whole-brain group x time interaction analysis. Finally, we explored associations between the evolution of connectivity and changes in cognitive performance. The early phase of mTBI was characterized by a functional hypoconnectivity in a subnetwork with a large overlap of regions involved within the classical default mode network. In addition, structural hyperconnectivity in a subnetwork including central hub areas such as the cingulate cortex was found. The impaired functional and structural subnetworks were strongly correlated and revealed a large anatomical overlap. One year after trauma and compared to healthy controls we observed a partial normalization of both subnetworks along with a considerable compensation of functional and structural connectivity subsequent to the acute phase. Connectivity changes over time were correlated with improvements in working memory, divided attention and verbal recall. Neuroplasticity-induced recovery or compensatory processes following mTBI differ between brain regions with respect to their time course and are not fully completed 1 year after trauma.

### 4.1.2 INTRODUCTION

Mild traumatic brain injury (mTBI) has an annual incidence of 100–300 cases per 100,000 persons, if counting only patients treated in hospitals (Cassidy et al., 2004). Although the majority of patients have a spontaneous history of favorable remission, mTBI can lead to long-term symptoms characterized by cognitive, emotional and physical disturbances. These symptoms are referred to as post-concussion disorder (PCD). It is increasingly being recognized that conventional diagnostic neuroimaging and measures of cognitive function are not suitable to predict outcomes and neuronal compensation after mTBI. Graph theoretical analysis supplies a straightforward way to evaluate complex neuronal networks (Bullmore and Sporns, 2009; Zalesky et al., 2010) as well as changes in connectivity following disrupted neuronal systems (Nakamura et al., 2009). In this respect, neuroimaging studies that combine functional and structural investigations of neuronal networks provide a more comprehensive picture of the neuroplasticity after mTBI. Furthermore, there have been only a few multimodal imaging studies aimed at elucidating the transitions between early and later stages of mTBI compared to the course of the healthy brain. Longitudinal magnetic resonance imaging (MRI) studies focusing on connectivity changes after mTBI have already been described, but these reported mixed findings, investigated only one MRI modality and had short follow-up periods. For example, some restoration of network dysfunction has been described over a period of 6 months after mTBI using longitudinal resting state fMRI within a specific frequency range in the default mode network (DMN) (Sours et al., 2015a). During the same time window, another study revealed recovery from diffuse decreased connectivity in the acute phase after injury, with the majority of the changes seen between 3 and 6 months and not between 0 and 3 months (Bharath et al., 2015). A trend of slow normalization within the DMN was even reported in a small pilot study with concussed athletes from day 7 to day 30 compared with the control group (Zhu et al., 2015). Mainly due to the very limited number of longitudinal connectomic studies, the relationship between restored functional connectivity over time and the corresponding changes in measures of cognitive functioning is just beginning to be explored (Bharath et al., 2015). On the contrary, other longitudinal studies did not reveal any functional recovery of initially decreased connectivity within the DMN and greater connectivity between the DMN and the prefrontal cortex (PFC) across a 4-month (Mayer et al., 2011) or a 6-month recovery period (Sours et al., 2015b).

In the existing mTBI literature, we did not identify studies looking at the longitudinal reorganization of structural network topology. Complementary, classical diffusion tensor imaging (DTI) studies reported about equal evidence of both increased and decreased

fractional anisotropy (FA) in adult samples during the semi-acute phase (Dodd et al., 2014). However, when focusing on studies conducted in acute mTBI rather increased FA has been revealed, while decreased FA findings are reported more frequently for post-acute studies (Eierud et al., 2014).

Studies of functional and structural connectivity changes during recovery after moderate and severe traumatic brain injury (TBI) are also relatively sparse and recent. By examining regional changes in the DMN, functional alteration has been demonstrated during a recovery period of 6 months after severe TBI (Hillary et al., 2011). The patients improved their performance on a working memory task and showed increased connectivity in the posterior cingulate cortex (PCC) and medial PFC, regions associated with internal-state responsivity. Another study on severe TBI aimed at delineating the progression of traumatic axonal injury in major fiber tracts over 6–11 months post-injury found that tractography-based measurements, which improved, remained stable, or deteriorated further, correlated with patients' long-term outcome ranging from good recovery to severely disabled (Wang et al., 2011). Nevertheless, all investigated white matter tracts showed systematic deterioration at group level.

We conducted a 1-year prospective study that tracks large-scale functional and structural network alterations in a cohort of mTBI patients compared to healthy controls. In addition, we explored how alterations of functional and structural connectivity are related to each other and how they are related to changes in cognition. A final objective of the current study was to determine whether mTBI patients with PCD would exhibit a different recovery trajectory in functional and structural connectivity, when compared with those without PCD.

### 4.1.3 MATERIALS AND METHODS

#### Participants: demographic and clinical data

A total of 60 patients with mTBI and 58 healthy controls matched for gender, age and education were prospectively recruited between February 2012 and March 2014. Healthy subjects were recruited through public advertising and among acquaintances of researchers and staff. The diagnosis of mTBI was given in accordance with the EFNS guidelines (Vos et al., 2012) using standardized criteria across the emergency departments of four hospitals in the German region of Switzerland. Inclusion criteria comprised (1) a GCS score of 13–15 at hospital admission; (2) a normal cranial CT; (3) at least one of the following characteristics: loss of consciousness < 30 min; presence of a qualitative alteration in mental status such as confusion, disorientation, or dizziness at the time of incident; post-traumatic amnesia < 60 min; and retrograde amnesia < 30 min; and (4) age ranging between 18 and 64 years. Exclusion

criteria were as follows: history of neurologic or psychiatric disease, neurosurgery, previous TBI, attention-deficit/hyperactivity disorder (ADHD,) current or previous drug or alcohol abuse, and contraindications to MRI. A previous mTBI in the preceding 3 months before investigation was an exclusion criterion. Nine patients and five controls were excluded because of incidental brain anomalies (two patients and one control), excessive MRI-related motion artifacts (two patients), history of prior neurologic or psychiatric disturbance (two patients and three controls), questionable diagnosis of mTBI (one patient) and uncertain follow-up participation (two patients and one control). This resulted in a sample of 51 patients and 53 controls. Patients were investigated clinically and brain images were acquired within 7 days post-injury (Visit 1, acute phase) and reinvestigated 1 year later (Visit 2, referred to as chronic phase). Within the same time interval, control subjects completed the identical assessments as the patients, expect for the neurological examination carried out by neurologists. Only participants who completed the entire investigation process across the two visits and were free of structural anomalies on conventional MR images were admitted for the longitudinal analysis reported here. Of the initial sample, 49 patients and all 53 controls returned for the follow-up visit (one patient moved abroad and another one was pregnant at Visit 2). To guarantee an exact one-to-one matching of patients and controls, the size of the control cohort was further restricted to 49 subjects. The four artificially excluded healthy subjects were representative of our healthy control group. The average time interval between scans was 365.9 days (SD = 4.0 days) for the patients as well as 364.6 days (SD = 5.1 days) for the controls. All subjects were equally reimbursed to make up for income lost due to study participation. Two Swiss Cantonal Ethics Committees (Ethics Committee Zurich and Ethics Committee for Northwest/Central Switzerland) approved the study protocol and all participants provided written informed consent prior to their participation.

## Neuropsychological assessment

A full battery of standardized and validated neuropsychological tests designed to be sensitive to diverse cognitive impairments commonly observed following mTBI was applied. These tests included measures of attention, executive functions, working memory, verbal memory as well as intelligence and measures of effort. In addition, clinical questionnaires were used to assess mTBI-related symptomatology as well as to control reactive manifestations of depression and anxiety. The descriptions of cognitive tests and clinical questionnaires are provided in the Supplementary Material (see A.1). A PCD was established at Visit 2 based on subjective symptoms from the Rivermead Post-Concussion Symptoms Questionnaire (RPQ)

(King et al., 1995). The RPQ assesses the most common PCD symptoms spanning cognitive, emotional and physical domains. At Visit 2, patients reporting three or more post-concussion symptoms rated as moderate to severe problems were pooled into a patient subcohort experiencing chronic PCD.

## Image acquisition protocols and preprocessing

Recordings included the following sequences: (a) resting-state T2*-weighted fMRI, (b) volumetric 3D T1-weighted MRI, (c) diffusion-weighted spin echo-planar imaging, (d) T1-weighted B0 map, (e) susceptibility-weighted imaging, (f) dual spin-echo (T2- and proton-density-weighted) as well as (g) fluid attenuated inversion recovery scans. Standard clinical MRI scans were evaluated at both time points by the same certified radiologist in order to detect intraparenchymal pathology (e.g., hemorrhages, tumors) considered as exclusion criteria. Details of functional and structural MRI data acquisition parameters are completely reported in the Supplementary Material (see A.1).

Preprocessing of rsfMRI and DTI data as well as the construction of functional and structural connectivity networks are described in detail in the Supplementary Material (see A.1).

## Statistical analyses

### Functional and structural connectivity analyses

Connectivity analyses were based on a 90-node whole-brain network constructed from the 90-region automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002), one node for each region of interest (ROI). Concerning functional connectivity, for each region a mean resting state time series was calculated as the mean over all voxel-time series of the respective region (mean ROI-time series). Functional connectivity measures are partial correlations accounting for white matter, cerebrospinal fluid, global signal, and for 24 head motion parameters (Friston-24). Fisher's z-transformed Pearson's correlation coefficients of mean ROI-time series pairs were taken as connectivity measure. For structural connectivity analysis, the numbers of streamlines connecting each pair of ROIs were used as connectivity measures. These values in form of connectivity matrices were subject to network-based statistic (NBS). NBS is a validated non-parametric method and a tool for identifying statistically significant subnetworks of a given network (connectivity matrix) (Zalesky et al., 2010). Using a family-wise error rate control with $p < 0.05$, NBS accounts simultaneously for multiple hypotheses testing over all edges of a network. To explore the potential linkage

between number of streamlines and more traditional diffusion measures, mean FA (averaged across all streamlines connecting two ROIs) of the subnetwork of interest was also calculated. Group comparisons at Visit 1 and interaction analyses were computed directly with NBS with the component *extent* option, significance level alpha = 0.05 corrected for multiple comparisons, one-sided hypothesis testing and 5,000 permutations per statistical test. The reported t-thresholds are sensitivity (set) thresholds (Zalesky et al., 2010) and were used to determine which edges of the connectivity matrix form the largest subnetwork subjected to permutation statistics. These thresholds have to be determined by exploration and are chosen in an arbitrary way. Specifically, the set t-threshold does not affect the false positive rate of the actual permutation statistic of the alpha error probability. Results of the network analyses were visualized using the BrainNet Viewer software (Xia et al., 2013). In NBS, we firstly ran a two-sample t-test for each of the possible 4,005 (90 x 89/2) connections to capture significant whole-brain difference in functional and structural connectivity between the patient and control groups at Visit 1. We then analyzed group x time interaction using NBS selecting exclusively the subnetwork identified as significant from the differences observed between groups at Visit 1 (selective interaction). For this purpose, we subtracted the Fisher's r-to-z-transformed Pearson's correlation coefficients (functional connectivity) and the number of reconstructed streamlines (structural connectivity) at Visit 2 from those at Visit 1 each resulting in a difference map operationalizing the change over time. If the interaction analyses resulted in significant subnetworks, we exported the data to IBM SPSS statistics software (22.0) and ran 2 (group) x 2 (time) repeated measures analysis of variances (ANOVAs) on connectivity values averaged across all edges of the significant subnetwork. In this way, we uncovered the direction of the interaction within the identified subnetworks as well as the main effects (between and within groups). The selective approach chosen here has the advantage of tracking the impaired subnetwork found at Visit 1 but has the disadvantage of overlooking possible differences not present within the first few days after mTBI. Therefore, we also assessed whole-brain group x time interactions for both functional and structural connectivity, independently from the initial level of network impairment. Finally, functional group differences (mean functional connectivity strength over a significant functional subnetwork) were correlated with structural group differences (mean structural connectivity strength over a significant structural subnetwork) at Visit 1 using Pearson's correlation. Additional correlations were calculated between functional and structural changes resulting from the selective group x time interactions. These post hoc analyses were performed using SPSS and a significance level of 0.05 (two-sided, unless otherwise indicated) was applied. The effect sizes

for group comparisons were computed according Cohen's d along with their associated 95% confidence intervals (CIs) and the effect sizes for group x time interactions were computed using partial eta-square and then converted to Cohen's d using formula reported elsewhere (Cohen, 1988). Cohen suggested defining the effect size as small (d = 0.2), medium (d = 0.5), and large (d = 0.8). Within- and between-group comparisons of the head motion parameters have been performed with t-tests using SPSS.

**Associations between cognitive performance and network metrics**

Cross-sectional differences in demographic and neuropsychological characteristics between groups were determined using unpaired t-tests and longitudinal differences within each group were performed using paired t-tests. Correlations between longitudinal changes in functional and structural mean connectivity strengths and longitudinal changes in cognitive performance were examined within the patients group only. These correlations were conducted using partial correlations controlling for age, education and total gray matter volume (for functional connectivity) or age, education and total white matter volume (for structural connectivity). All associations between cognitive performance and network metrics were run in SPSS with partial Spearman rank-order correlations (rho) that also account for the influence of outliers in the neuropsychological measurements.

### 4.1.4 RESULTS

## Participant characteristics and neuropsychological measures

Table 4.1.1 summarizes the characteristics of the patients (n = 49) and of the healthy controls (n = 49) included in the final sample. Note that there were no significant differences in potential demographic confounders or in the time interval of MRI acquisitions between the two groups.

## Table 4.1.1 Characteristics of patients and control groups

| | Visit 1 (acute phase) | | | Visit 2 (chronic phase) | | | Repeated measures | |
|---|---|---|---|---|---|---|---|---|
| | Patients (n = 49) mean (SD) | Controls (n = 49) mean (SD) | Pat vs. Con p-value | Patients (n = 49) mean (SD) | Controls (n = 49) mean (SD) | Pat vs. Con p-value | Patients p-value | Controls p-value |
| **Demographics and clinical measures** | | | | | | | | |
| Age (years) | 34.9 (12.4) | 35 (12.1) | 0.96 | 35.9 (12.4) | 36 (12.1) | 0.96 | n/a | n/a |
| Gender (male/female) | 18 / 31 | 18 / 31 | 1.00 | 18 / 31 | 18 / 31 | 1.00 | n/a | n/a |
| Education (years) | 12.6 (2.5) | 12.9 (2.4) | 0.49 | 12.7 (2.5) | 13 (2.5) | 0.44 | 0.02 | 0.01 |
| Days between scans | - | - | - | 365.9 (4.0) | 364.6 (5.1) | 0.18 | - | - |
| Glasgow Coma Scale | 14.8 (0.4) | - | - | - | - | - | - | - |
| MTBI in the past | 0.6 (0.9) | 0.4 (0.8) | 0.18 | - | - | - | - | - |
| **Global brain measures** | | | | | | | | |
| Total grey matter volume ($cm^3$) | 650.4 (67.3) | 649.1 (55.3) | 0.92 | 649.5 (66.5) | 646.7 (54.4) | 0.82 | 0.71 | 0.15 |
| Total white matter volume ($cm^3$) | 474.1 (58.8) | 478.3 (38.4) | 0.68 | 474.8 (59.6) | 477.7 (37.7) | 0.77 | 0.43 | 0.39 |
| **Global connectivity measures (90 nodes)** | | | | | | | | |
| Number of streamlines | 2,159,848 | 2,147,650 | 0.78 | 2,158,364 | 2,162,031 | 0.93 | 0.89 | 0.08 |
| Streamlines omitted | 1,375,985 | 1,353,384 | 0.39 | 1,368,688 | 1,362,667 | 0.81 | 0.41 | 0.17 |
| Streamlines used to populate matrix | 832,699 | 840,888 | 0.75 | 837,946 | 846,829 | 0.73 | 0.55 | 0.39 |
| Selfloops | 468,174 | 464,983 | 0.79 | 468,230 | 466,147 | 0.86 | 0.99 | 0.65 |
| **Neuropsychological assessment** | | | | | | | | |
| RPQ (total score) | 14.2 (10.8) | 2.8 (3.9) | <0.001 | 7 (9.9) | 2.5 (4.4) | 0.005 | < 0.001 | 0.54 |
| Alertness, tonic (ms) | 243.2 (53.4) | 220.4 (20.5) | 0.007 | 219.2 (20.9) | 216.9 (20.2) | 0.59 | 0.004 | 0.16 |
| Alertness, phasic (ms) | 244.7 (70.2) | 221.4 (22.8) | 0.03 | 215.9 (18.1) | 216 (21.2) | 0.99 | 0.01 | 0.10 |
| Go/Nogo (ms) | 398.3 (69.1) | 370.1 (45.2) | 0.02 | 380.4 (62.2) | 359.2 (46.1) | 0.06 | 0.02 | 0.04 |
| Go/Nogo (errors) | 0.9 (1.2) | 1.0 (1.1) | 0.61 | 0.9 (0.9) | 0.9 (0.9) | 0.83 | 0.73 | 0.45 |
| Divided attention, auditory (ms) | 584.9 (94.4) | 559.6 (75.4) | 0.15 | 553.8 (70.9) | 532.6 (68.2) | 0.13 | 0.03 | 0.01 |
| Divided attention, visual (ms) | 805.9 (105.1) | 756.9 (89.2) | 0.01 | 741.7 (90.7) | 706.2 (82.5) | 0.05 | < 0.001 | < 0.001 |
| Working memory | 5.4 (1.2) | 5.8 (1.3) | 0.05 | 5.9 (1.4) | 6 (1.2) | 0.64 | 0.001 | 0.39 |
| AVLGT immediate recall score | 57.5 (6.9) | 59.4 (6.9) | 0.18 | 61.9 (6.1) | 63.3 (6.8) | 0.33 | < 0.001 | < 0.001 |
| AVLGT long delayed | 12.9 (1.8) | 13.2 (1.9) | 0.48 | 13.9 (1.6) | 14.1 (1.2) | 0.48 | 0.002 | < 0.001 |
| BDI-II (score) | 6.4 (5.8) | 3.5 (4.4) | 0.01 | 4.1 (4.8) | 2.8 (4.3) | 0.17 | 0.003 | 0.28 |
| BAI (score) | 2.8 (6.6) | 0.6 (2.2) | 0.03 | 1.9 (4.5) | 0.14 (1.0) | 0.01 | 0.40 | 0.04 |
| Intellectual ability (IQ) | 101.2 (15.1) | 107.8 (14.5) | 0.03 | - | - | - | - | - |
| Performance validity (MSVT) | - | - | - | Good effort | Good effort | | | |

n/a = not applicable; RPQ = Rivermead Post-Concussion Symptoms Questionnaire; AVLGT = German adaptation of the Rey Auditory Verbal Learning Tests RAVLT; BDI-II = Beck Depression Inventory, 2nd edition; BAI = Beck Anxiety Inventar; MSVT = Medical Symptom Validity Test.

The mean age of the patients was 34.9 years (range 18–61 years) and that of the controls was 35 years (range 18–60 years). At arrival in the emergency department, the majority of the patients (n = 40) had an initial GCS of 15, eight patients were admitted with 14 and one patient with 13. Neuroimaging and neuropsychological investigations were performed at an average of 4.9 days (SD = 1.5 days) and 5.3 days (SD = 1.6 days) post-injury, respectively. Compared to the well-matched control group, patients with mTBI showed initial worse performance across a subset of cognitive tasks and greater impairments on questionnaire-based clinical measures. In particular, patients exhibited significantly higher symptom severity on the RPQ. At follow-up, cognitive recoveries as well as clinical improvements have been observed, although higher scores of post-concussive symptoms were still measured in patients. Six of 49 patients were identified as having a chronic PCD (about 12%) on the basis of their symptoms reported 1 year after the injury and the remaining 43 patients had a good outcome. We did not observe any differences in age (p = 0.19), sex (p = 0.65), and GCS (p = 0.83) between the patients with and without chronic PCD. However, there was a statistical trend in the years of education (mean/SD: 12.84/2.5 years for good outcome, 10.83/1.5 years for poor outcome, p = 0.06) with chronic PCD-patients having fewer years of schooling. All participants showed good effort on tests of symptom validity. The corrected p-values for all 49 tests adjusted using false discovery rate (FDR) correction are reported in Supplementary Table A.1.

There were no statistically significant group differences in head motion parameters of both the functional and structural MRI scans neither between groups at both time points nor between time points within groups. For the rsfMRI data, mean frame-wise displacements computed according the method proposed by Power (Power et al., 2012; Power et al., 2015) and for the DTI data, mean frame-wise translations and rotations computed according the method proposed by Yendiki (Yendiki et al., 2014) were small and comparable between groups at both time points and between time-points within groups.

## Functional and structural connectivity analyses – group comparison

At Visit 1, functional hypoconnectivity within a 15-edge subnetwork distributed over 15 nodes was detected in the mTBI group compared to controls (Cohen’s d = 1.59, 95% CI = 1.138–2.046, p = 0.0057). The pattern of this subnetwork with reduced functional connectivity consisted almost exclusively of bilateral structures (12 of 15 nodes) such as the anterior cingulate cortex (ACC), PCC, precuneus, Heschl's gyrus, superior temporal gyrus (STG) and temporal pole (TP). In addition, the parahippocampal gyrus, amygdala and supplementary

motor area (SMA) were only present in the right hemisphere (Fig. 4.1.1 and Supplementary Table A.2).

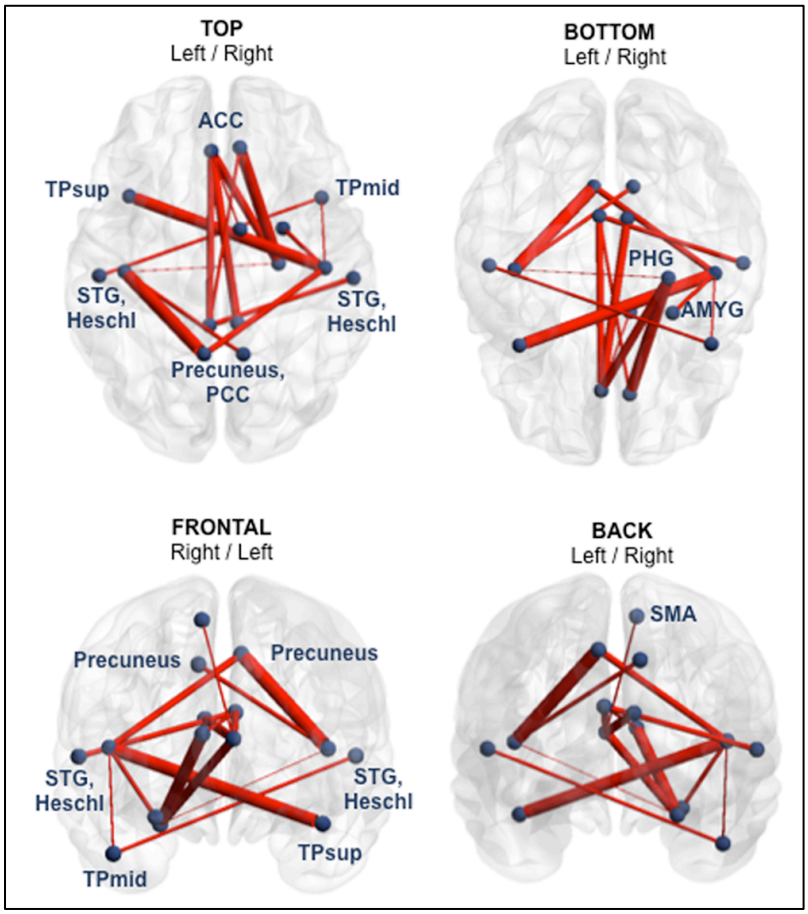


**Figure 4.1.1. Subnetwork with reduced functional connectivity in the mTBI sample at Visit 1 (group comparison)**

Blue points correspond to the 15 nodes of the subnetwork and red lines represent the 15 suprathreshold connections that showed reduced functional connectivity in mTBI patients, compared with healthy controls. Cohen's d = 1.59, 95% CI = 1.138–2.046, p = 0.0057, corrected for multiple comparisons. The NBS-specific set threshold forming the component was set to t = 3.16. More details about the connections of this subnetwork can be found in Supplementary Table A.2. Abbreviations: ACC = anterior cingulate cortex, AMYG = amygdala, PCC = posterior cingulate cortex, PHG = parahippocampal gyrus, SMA = supplementary motor area, STG = superior temporal gyrus, TPmid = middle temporal pole, TPsup = superior temporal pole

The profile of the altered edges showed 10 inter- and five intra-hemispheric connections. Of note, seven of the 15 edges involved the nodes ACC or PCC. Briefly, the impaired functional subnetwork revealed many key nodes of the classical DMN.

In the cross-sectional structural analysis, patients showed a widespread increase in connectivity compared to controls (Cohen's d = -1.71, 95% CI = -2.168 to -1.243, p = 0.041). Structural hyperconnectivity was detected in a 53-edge subnetwork distributed over 52 nodes comprising connections encompassing frontal, temporal, parietal, occipital, and subcortical regions (Fig. 4.1.2 and Supplementary Table A.3).

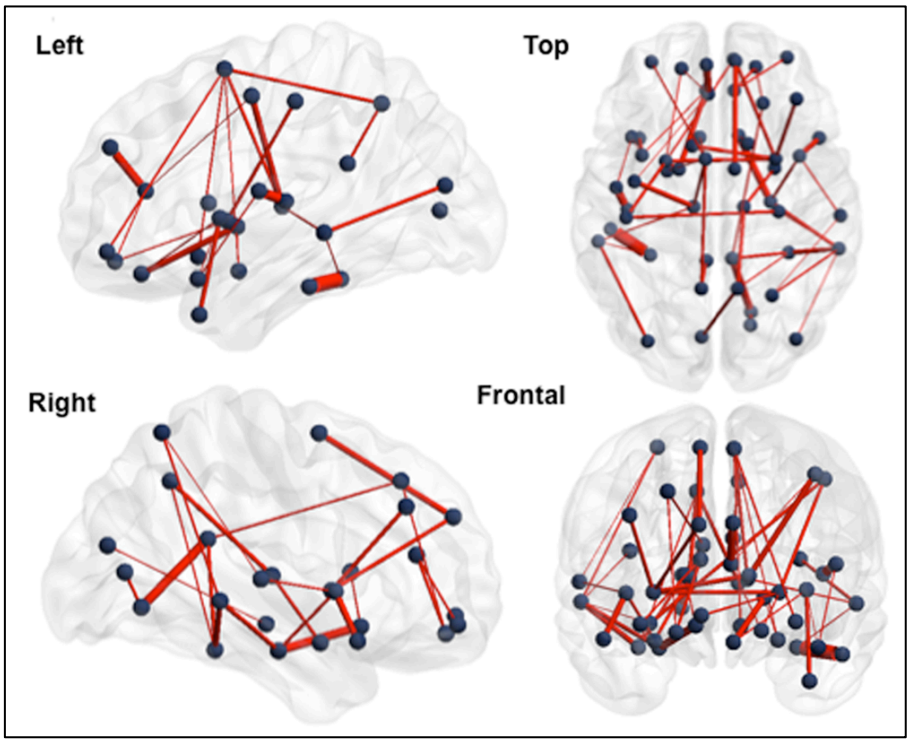


**Figure 4.1.2. Subnetwork with increased structural connectivity in the mTBI sample at Visit 1 (group comparison)**

Blue points correspond to the 52 nodes of the subnetwork and red lines represent the 53 suprathreshold connections that showed increased structural connectivity in mTBI patients, compared with healthy controls. Cohen's d = -1.71, 95% CI = -2.168 to -1.243, p = 0.041, corrected for multiple comparisons. The NBS-specific set threshold was set to t = 1.87. More details about the connections of this subnetwork can be found in Supplementary Table A.3.

Of the 53 edges, 48 were intra-hemispheric, whereas 36 of the 52 nodes were bilaterally represented, including ACC, PCC, precuneus and TP. This increase in the number of streamlines between these nodes in the mTBI group also corresponded to a significant increase in FA as well as to a decrease in global efficiency and an increase in normalized characteristic path length compared with healthy controls (see Supplementary Results A.2 and Supplementary Fig. A.1). No brain area showed functional hyperconnectivity or structural hypoconnectivity in patients relative to controls. We also estimated the reproducibility of the structural finding, which were obtained using the "eddy_correct" tool, with that obtained by employing the "eddy" tool for the correction of eddy currents including parallel the average of the mean of translational and rotational motion estimation parameters as a nuisance regressor in the analysis with NBS. A common artifact in diffusion imaging is signal attenuation caused by macroscopic head motion (Yendiki et al., 2014). We demonstrated reproducible mTBI-related group differences across the different corrections of eddy current-induced distortions (see Supplementary Results A.2 and Supplementary Fig. A.3–4). The fact that both results were qualitatively comparable may be attributable to the low b-value of 1,000 s/mm$^2$ (Andersson and Sotiropoulos, 2016).

## Functional and structural connectivity changes over time – interaction

The group x time interaction of the impaired subnetworks defined at Visit 1 (selective interaction) displayed significant results in both functional and structural connectivity. Firstly, a significant functional change was identified in the whole 15-edge subnetwork (Cohen's d = 0.9, 95% CI = 0.490–1.321, p = 0.002, Fig. 4.1.3A, Supplementary Table A.4).

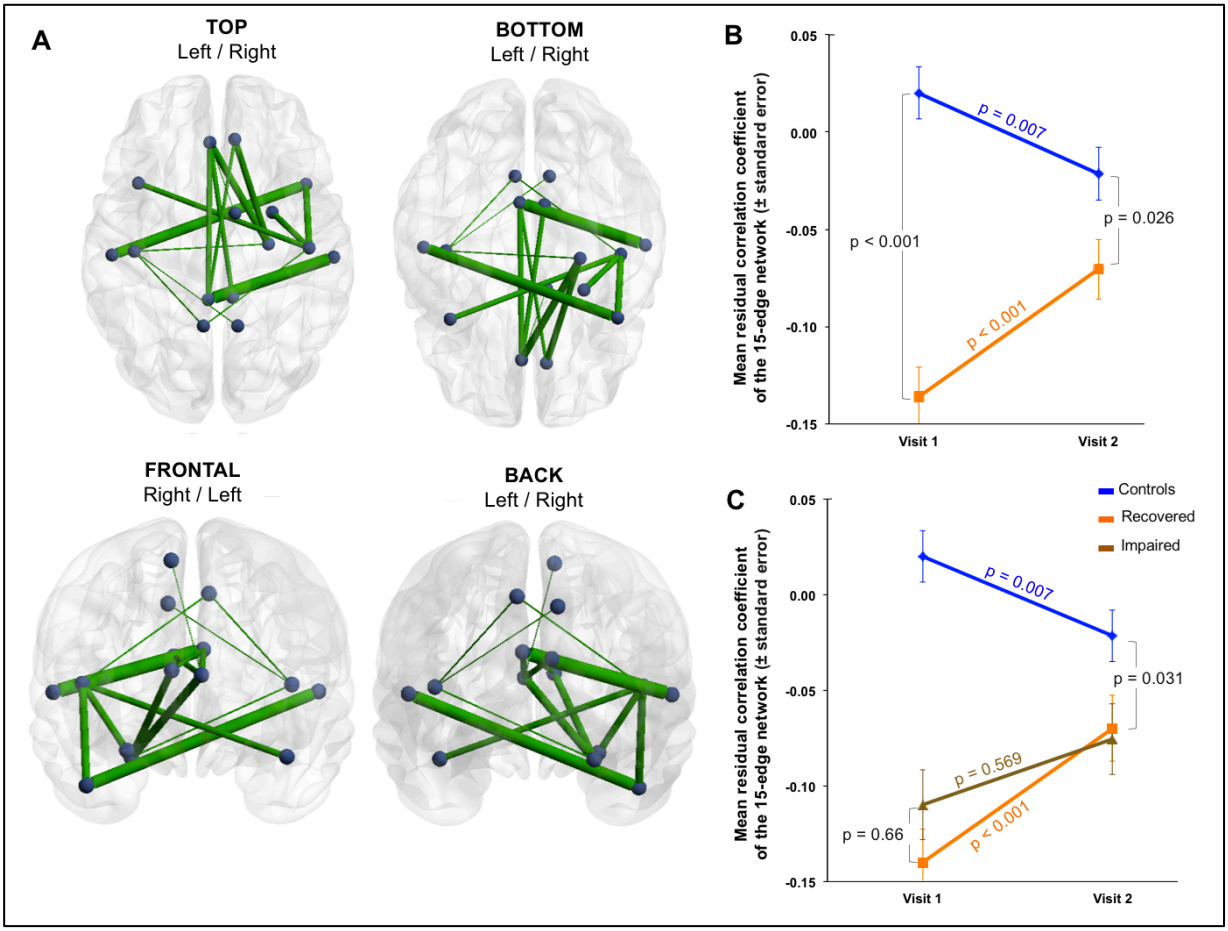


**Figure 4.1.3. Functional changes over 1 year within the initially impaired 15-edge subnetwork (selective group x time interaction)**

**(A)** The NBS-specific set threshold was set to t = 0 in order to admit all possible connections of the 15-edge subnetwork to the set of suprathreshold links showing a change over time (Cohen's d = 0.9, 95% CI = 0.490–1.321, p = 0.002, corrected for multiple comparisons). **(B)** The significant repeated-measures effect resulted from an increase in the mean correlation coefficient within the patient sample along with a decrease within the control sample. The time effect for each group revealed significant evolutions over 1 year. **(C)** The line graph is color-coded orange and brown corresponding to patients with good (n = 43) and poor outcome (n = 6), respectively. The group difference was smaller at 1-year post-injury when only the 43 patients with good outcome were considered. Repeated measure over the subset of patients with good outcome versus controls revealed a more rapid trajectory towards normalization. At a descriptive level, the recovery curves in patients developing chronic post-concussive syndrome were somewhat flat. More details about the connections of this subnetwork can be found in Supplementary Table A.4.

The exploration of this longitudinally altered subnetwork showed that an increase in the mean functional connectivity within the patient sample and a decrease within the control sample were responsible for the significant interaction observed (Fig. 4.1.3B). The group effect within the 15-edge subnetwork reached a marked difference at Visit 1 that shifted into a weaker, but still statistically significant difference at Visit 2. When only the 43 patients with GO were considered, the group difference at 1-year post-injury was smaller (Fig. 4.1.3C). Furthermore, the effect of time for each group separately showed significant evolution in the connections' strength. The recovery curve in patients with persistent PCD (n = 6) was rather flat compared with that of patients with GO (n = 43), even though statistical analysis on a small sample size should be interpreted with caution.

Secondly, the group x time interaction of structural connectivity within the 53-edge subnetwork resulting from the group comparison at Visit 1 (selective interaction) revealed significant alterations in two distinct subnetworks (Supplementary Table A.5). One subnetwork was concentrated in the frontal part of the left hemisphere and showed a profile of 19 edges distributed over 19 nodes (Cohen's d = -0.72, 95% CI = -1.132 to -0.315, p = 0.025, subnetwork 1A in Fig. 4.1.4).

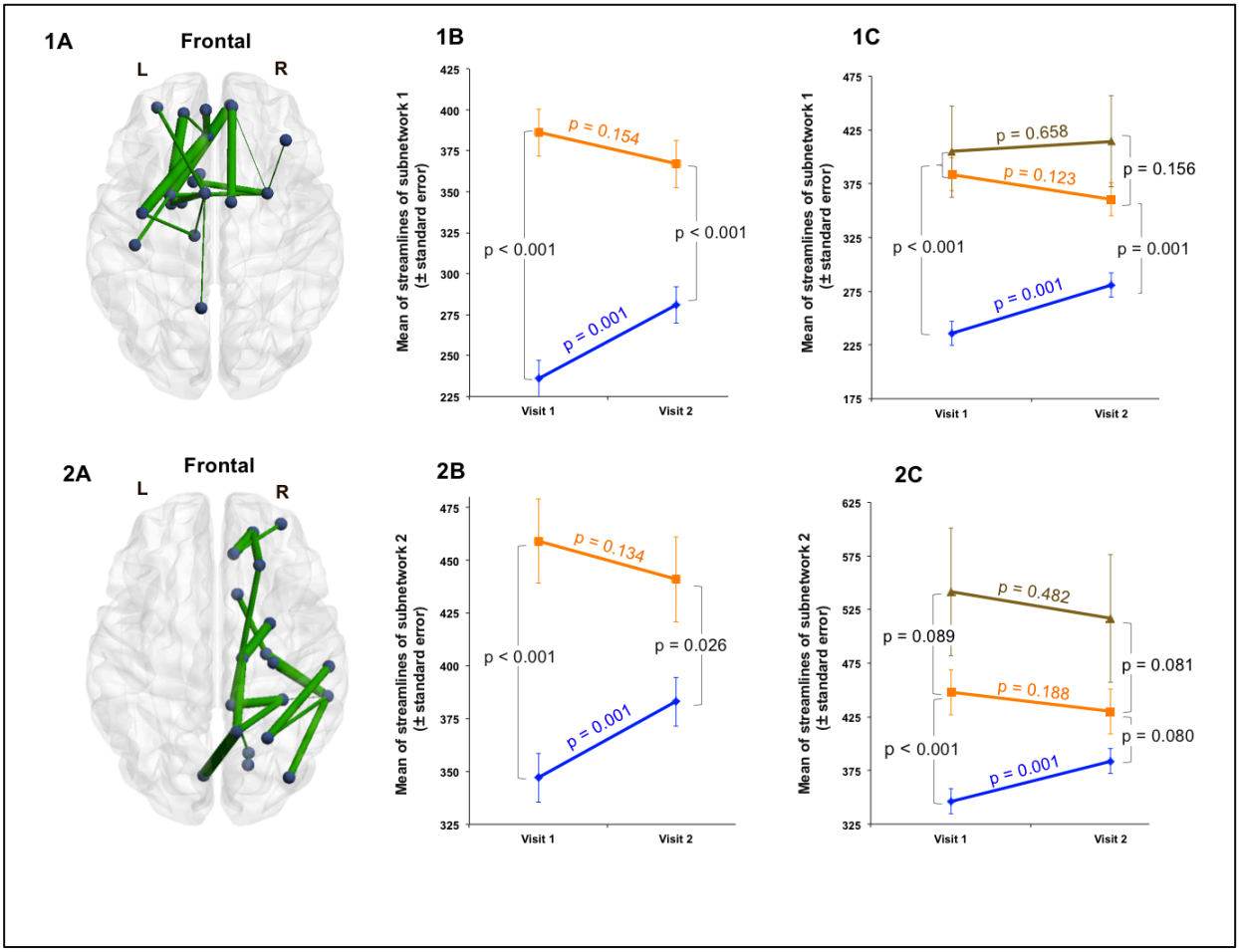


**Figure 4.1.4. Structural changes over 1 year within the initially impaired 53-edge subnetwork (selective group x time interaction)**

The NBS-specific set threshold was set to t = 0 in order to admit all possible connections of the 53-edge subnetwork to the set of suprathreshold links showing a change over time. **(1A)** Subnetwork 1 encompassed 19 edges and is accentuated toward the left hemisphere (Cohen's d = -0.72, 95% CI = -1.132 to -0.315, p = 0.025, corrected for multiple comparisons). **(2A)** Subnetwork 2 comprised 18 edges and is accentuated toward the right hemisphere (Cohen's d = -0.71, 95% CI = -1.120 to -0.303, p = 0.035, corrected). **(1B, 2B)** A decrease in the number of streamlines in the patient sample and an increase in the control sample were responsible for the significant repeated-measures effect. The time effect was significant in the controls, but only weakly trend in the patients **(1C, 2C)**. When focusing on the group analyses of the subcohort of patients without post-concussive syndrome (n = 43, orange trajectory) at follow-up, the difference to the control group was no longer significant for the 18-edge subnetwork, but still evident for the 19-edge subnetwork. There was no significant difference between patients with and without PCD at Visit 2. More details about the connections of this subnetwork can be found in Supplementary Table A.5.

The second subnetwork was concentrated in the right hemisphere and consisted of 18 connections distributed over 19 nodes (Cohen's d = -0.71, 95% CI = -1.12 to -0.303, p = 0.035,

subnetwork 2A in Fig. 4.1.4). The splitting into two subnetworks was due to the fact that only five of 53 connections composing the initially altered subnetwork were inter-hemispheric. Both structural subnetworks demonstrating changes over time revealed decreased connectivity for the patients along with increased connectivity for the controls (Fig. 4.1.4).

At the initial visit, the group effect was strong for both subnetworks. One year later, both structural subnetworks normalized in individuals with mTBI, but the recovery was not complete since the mean of streamlines was still increased compared to controls. No significant difference was found between patients with and without PCD at Visit 2. When focusing on the group analyses on the subcohort of patients without PCD at follow-up, the difference to the control group was no more significant for the 18-edge subnetwork, but still evident for the 19-edge subnetwork. The time effect in the patients with GO approached levels of a weak trend again, whereas the trajectories of patients with persistent PCD were rather flat for both subnetworks (Fig. 4.1.4). Please note, findings should be interpreted with caution due to the small number of patients with chronic PCD (n = 6). Other figures showing the functional and structural connectivity changes over time at individual level can be found in the Supplementary Figures A.5–7.

The group x time interaction analysis of FA indicated a trend towards decreased mean FA for the patients over time and is reported in the Supplementary Results A.2 (see also Supplementary Fig. A.2).

Finally, whole-brain group x time interactions for functional and structural connectivity were investigated. The functional subnetwork detected consisted of 59 edges distributed over 48 nodes and the structural subnetwork encompassed 1,023 edges distributed over all 90 nodes (Fig. 4.1.5, Supplementary Table A.6).

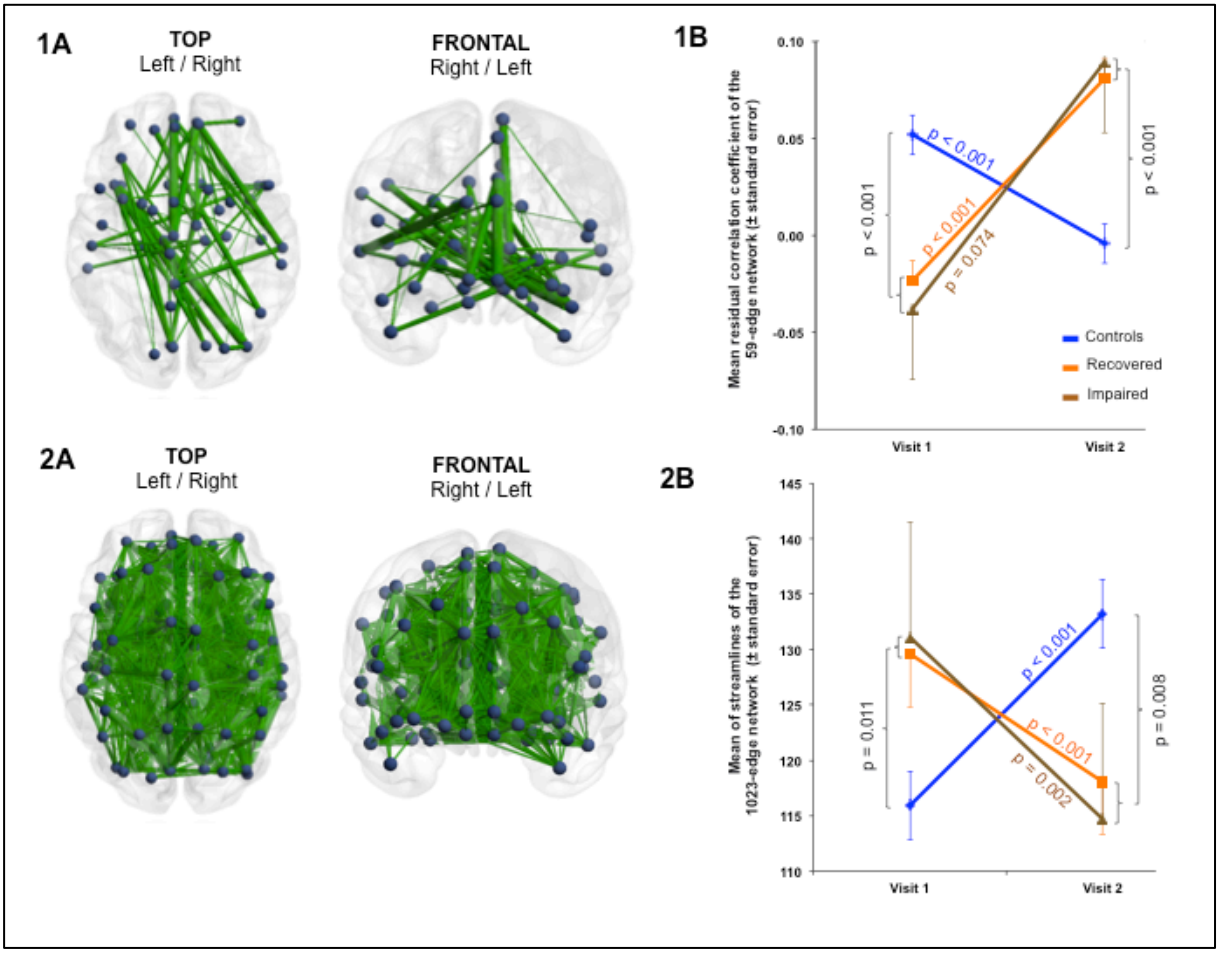


**Figure 4.1.5. Functional and structural large-scale changes over 1 year (whole-brain group x time interaction)**

The NBS-specific set threshold for the functional connectivity was set to t = 2.42, while that of the structural connectivity was set to t = 0.65. **(1A)** A functional subnetwork composed of 59 edges and 48 nodes was detected (Cohen's d = 1.87, 95% CI = 1.402–2.353, p = 0.045, corrected for multiple comparisons). **(2A)** A widespread structural subnetwork composed of 1,023 edges and 90 nodes was found (Cohen's d = -1.85, 95% CI = -2.325 to -1.378, p = 0.045, corrected). **(1B)** At the large-scale level, functional connectivity increased in mTBI patients over time, **(2B)** whereas structural connectivity decreased. The p-values in the figure refer to the group effect of the patients without chronic post-concussive syndrome plotted against the healthy controls. More details about the connections of the functional subnetwork can be found in Supplementary Table A.6. Due to the large number of connections affected, no table is provided for the structural subnetwork.

Although with more nodes and connections, similar scenarios as for the selective interactions were observed for the whole-brain interaction: functional connectivity increased (Cohen's d = 1.87, 95% CI = 1.402–2.353, p = 0.045) while structural connectivity decreased within the patients over time (Cohen's d = -1.85, 95% CI = -2.325 to -1.378, p = 0.045). In contrast to the partial recoveries of the functional and structural connections found in the

selective interactions, whole-brain interactions revealed a steeper recovery trajectory in the patients (functional and structural time effect $p < 0.001$) independent of the severity of symptoms at Visit 2 (Fig. 4.1.5). Neither the structural nor the functional connectivity alterations revealed by the selective as well as whole-brain interactions were statistically significantly correlated with age, for both across and within groups.

### Function-structure overlapping

Looking at the anatomical patterns of the brain subnetworks described above, lots of impaired but also recovered nodes of the functional and structural connectivity analyses were exactly the same. At Visit 1, the common damaged nodes encompassed bilateral ACC and PCC, precuneus, right STG, right SMA, right parahippocampal gyrus, right amygdala as well as left Heschl's gyrus and left TP. Of these, all structures except for left PCC, left Heschl's gyrus and left STG were also involved in the post-injury recovery phase (Fig. 4.1.6).

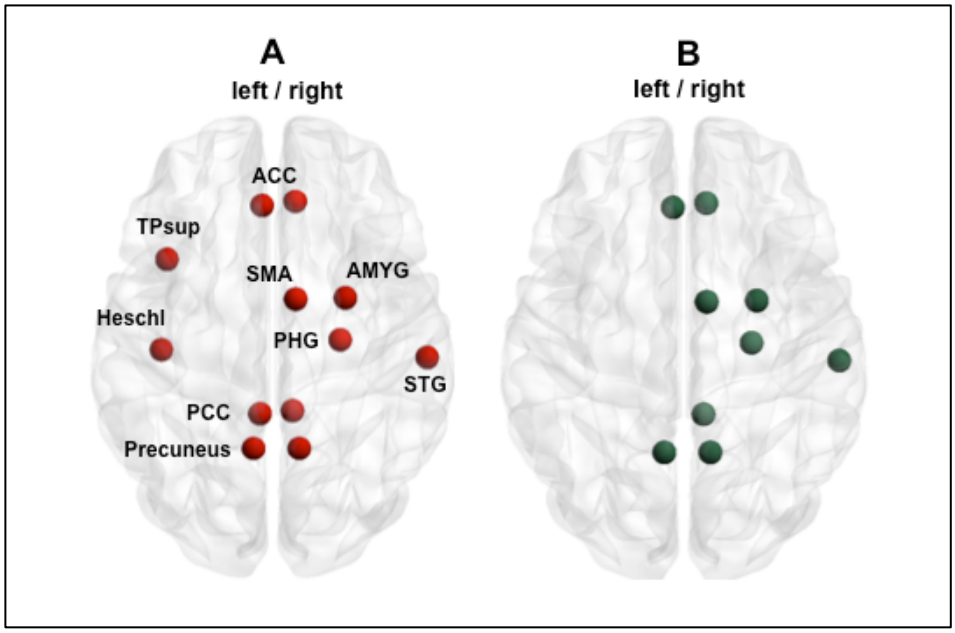


**Figure 4.1.6. Function-structure overlapping**

**(A)** Red points illustrate nodes impaired acutely after mTBI in both functional and structural connectivity analysis (group comparison at Visit 1). **(B)** Green points display partially recovered nodes over time at both functional and structural level (selective group x time interaction). Abbreviations: ACC = anterior cingulate cortex, AMYG = amygdala, PCC = posterior cingulate cortex, PHG = parahippocampal gyrus, SMA = supplementary motor area, STG = superior temporal gyrus, TPsup = superior temporal pole

Informed by functional hypoconnectivity and structural hyperconnectivity in the patients at Visit 1 and in accordance with observations from studies with moderate/severe TBI that detected more white matter disruption in patients with less functional connectivity within the DMN (Sharp et al., 2011), we hypothesized a relationship between the structurally and functionally impaired subnetworks. Indeed, we found a negative correlation between the strength of the functional with that of the structural subnetwork at Visit 1 ($r = -0.243$, $p = 0.046$, one-sided) in the group of mTBI patients, but not in controls ($r = -0.088$, $p = 0.547$). Hence, we observed that the stronger the hypoconnectivity in the functional circuits the stronger the hyperconnectivity in the structural circuits.

Finally, we tested the possibility that functional connectivity reorganizations over time were affected by underlying structural reorganizations by means of restoration of diffuse axonal injuries. No significant correlations were found between longitudinal changes in the functional network with those in the structural network, neither for the selective nor for the whole-brain interaction analyses.

## Relationship between network and cognition over time

Relationships between alterations in mean connectivity (functional and structural) and in cognitive performance across time points were assessed within the mTBI sample only. Here, the results are reported one-sided since we expected that long-term alterations in connectivity would be associated with the – in the literature widely reported – cognitive improvement over time. We found significant correlations between functional recovery and performance improvement in a working memory test ($rho = -0.350$, $p = 0.008$) as well as in the speed of a divided attention task of visual stimuli ($rho = 0.333$, $p = 0.012$, Fig. 4.1.7A). Decrease in functional connectivity strength of the 15-edge network, reflecting the ability to deactivate this DMN-like network, was associated with improvement in the performances of two executive tasks.

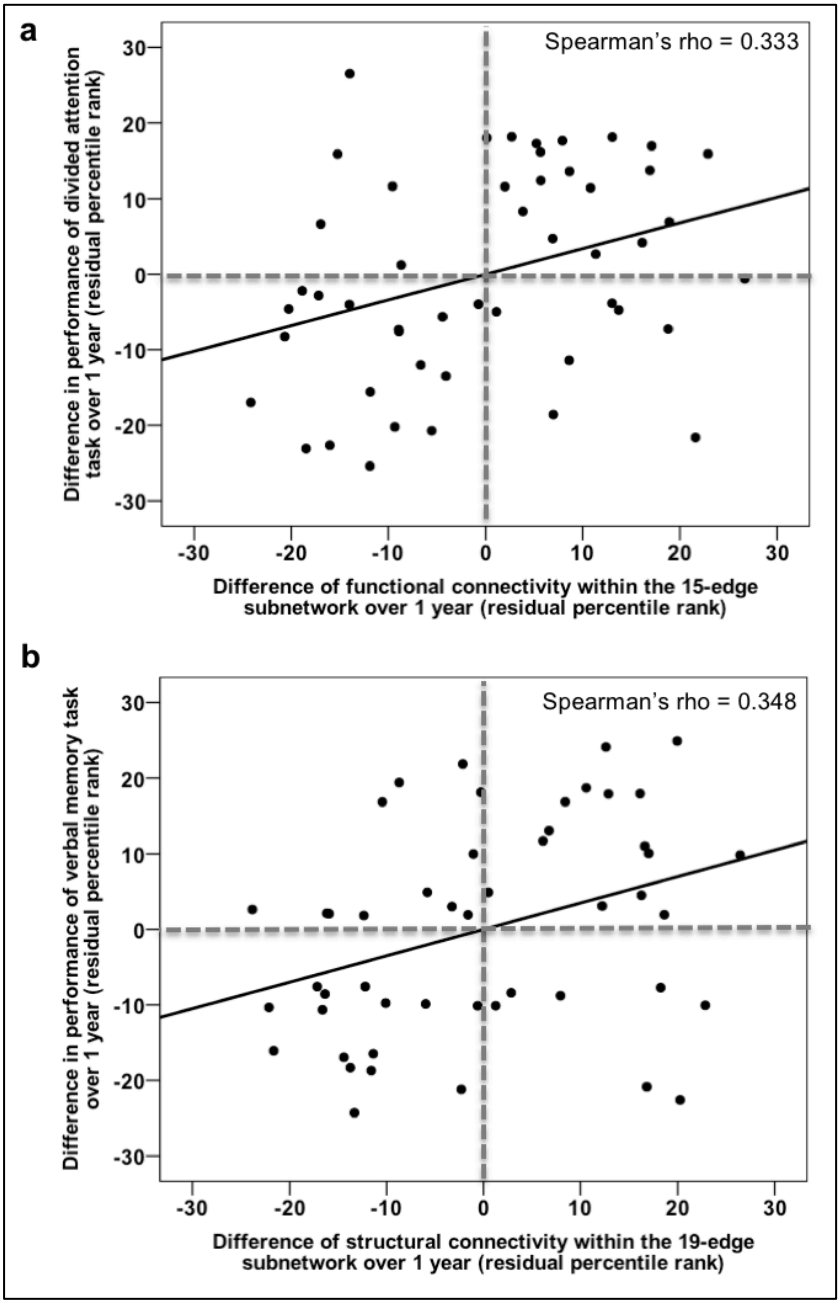


**Figure 4.1.7. Recovery in functional and structural connectivity of the initially impaired subnetworks correlates with task improvement over 1-year post-injury**

**(A)** Changes in mean functional connectivity within the default mode-like 15-edge subnetwork are plotted against changes in median reaction time on the divided attention task. **(B)** Changes in mean structural connectivity within the 19-edge subnetwork are plotted against changes in recalled items on the verbal memory task.

In addition, we identified a positive correlation between structural connectivity changes and improvement in a long-delayed recall test of verbal memory (rho = 0.348, p = 0.009, Fig. 4.1.7B) as well as in a divided attention task (rho = -0.262, p = 0.039). The observed correlations were however, not corrected for multiple testing. After the FDR adjustment tested across 30 correlations [product of 10 test scores and (i) the recovered functional subnetwork as well as recovered structural subnetworks 1 (ii) and 2 (iii), respectively], uncorrected significances did not survive the FDR correction (Supplementary Table A.7). No correlations were found in patients neither for any other neuropsychological assessments nor for the clinical measurements.

## 4.1.5 DISCUSSION

The present study tracks, for the first time to the best of our knowledge, large-scale dynamics of resting-state functional and DTI-based structural connectivity over a period of 1-year following mTBI in a relatively large sample of 49 mTBI patients and 49 controls. We revealed three main findings: (i) The acute scenario of the injured brain started with functional hypoconnectivity in a subnetwork broadly analogous to the classical DMN and structural hyperconnectivity in a subnetwork involving widespread brain areas. The initially impaired functional and structural architectures were not only inversely related to each other, but also revealed a considerable anatomical overlap. (ii) Longitudinally, we demonstrated a partial recovery of both subnetworks disturbed at Visit 1, along with additional, considerable compensation of functional and structural connectivity patterns altered subsequent to Visit 1. (iii) We provided evidence that connectivity changes over time were clinically relevant.

### Alterations in functional and structural connectivity in the acute phase

Considering the lack of consensus about the location of mTBI-induced brain alterations and the distributed effect of diffuse axonal injuries on the brain, we employed a whole-brain rather than a seed-based hypothesis-driven approach.

#### Functional connectivity

Firstly, the functional analysis detected a subnetwork of reduced connectivity in patients composed of 15 connections and 15 nodes. Of these, 11 nodes are known to be part of the DMN, namely bilateral PCC, ACC, precuneus, STG, TP and the right parahippocampal gyrus (Greicius et al., 2003; Buckner et al., 2008). Furthermore, this subnetwork, composed of mostly inter-hemispheric connections, exhibited four additional nodes arranged outside the

DMN components, i.e. bilateral Heschl's gyrus, right SMA and right amygdala. The pattern of this DM-like subnetwork differed from the pattern of the classical DMN, particularly due to the absence of the medial PFC that, together with the PCC, reflect the core set of hubs within the DMN (Andrews-Hanna et al., 2010). However, our findings are in accordance with previous mTBI studies that also observed a shift of the DMN towards functional hypoconnectivity within 7 days (Iraji et al., 2015; Zhu et al., 2015), within 11 days (Sours et al., 2015b) and in the semi-acute stage post-injury (Mayer et al., 2011; Johnson et al., 2012). Some of these studies did not focus exclusively on connectivity within the DMN and also demonstrated increased connectivity between DMN and task-positive networks, or among other brain regions (Mayer et al., 2011; Iraji et al., 2015). One reason why our results did not reveal functional hyperconnectivity between multiple networks may arise from the different method used to determine the network of interest (ROI-based versus whole-brain approach). However, other studies examining whole-brain functional connectivity by means of independent component analysis, which is not biased by a priori assumptions, detected profiles of decreased as well as increased connectivity in the same sample of semi-acutely injured mTBI patients (Shumskaya et al., 2012; Stevens et al., 2012).

**Structural connectivity**

Secondly, the structural analysis showed increased connectivity for patients in a 53-edge subnetwork composed of mainly intra-hemispheric connections and bilateral structures. In part, these structures were already detected in the functional connectivity analysis, including bilateral ACC, PCC, precuneus, STG, TP and right parahippocampal gyrus. The 53-edge subnetwork revealed increased connectivity of central hub areas comprising superior frontal cortex, superior parietal cortex, precuneus and subcortical putamen, thalamus and hippocampus. These specific hub regions were found to be more densely connected among themselves than to other regions of the human connectome, suggesting a "rich-club" organization (van den Heuvel and Sporns, 2011). Highly connected central hubs of the brain are known to be vulnerable targets susceptible to disturbance in neurological disorders such as Alzheimer's disease (Stam et al., 2009) as well as fundamental for multiple cognitive functions (van den Heuvel et al., 2009b). Surprisingly, our findings detected alterations in these densely connected network hubs, for example, the PCC and ACC, and interestingly it has been reported that these are affected in moderate/severe TBI too (Sharp et al., 2011; Pandit et al., 2013; Sharp et al., 2014). This suggests an analogy between the pathomechanisms of mTBI and that of TBI. Stam considered the selective vulnerability of hub regions with the mechanism of "hub

overload" followed by "hub failure" (Stam, 2014). In his model, the healthy neuronal network is illustrated as a hierarchical tree, where nodes at the lowest level could represent primary sensory and motor regions, while hubs at subsequent higher levels may represent multi- or supramodal association areas. Disruption of some nodes diminished their ability to handle incoming information, resulting in information traffic being rerouted to nodes higher up in the hierarchy. As a result, the traffic load of higher nodes increases and is redirected again to nodes even higher up, until the highest hubs are reached. This mechanism provokes a hub overload (Stam, 2014). Our findings of increased structural connectivity in the acute phase can therefore be explained with a rerouting of damaged nodes to the highest hubs. To directly compare our data with studies reporting traditional DTI parameters, we also explored FA values within the affected subnetwork. The increased number of streamlines reflected increased FA and this is further supported by a strong positive correlation between the two measures. In the extensive DTI literature and as recently reviewed, higher FA is not uncommon when focusing on the acute mTBI stage (Dodd et al., 2014; Eierud et al., 2014; Pacifico et al., 2015).

**Functional–structural association**

Next, we observed a functional-structural relationship between the altered connectivity patterns. The functionally hypoconnected and the structurally hyperconnected subnetwork displayed 12 nodes, but no connections in common. At first glance, these two findings might appear contradictory. On the one hand, there is no function-structure overlap at the connection level, leading to the interpretation that there is no association between disturbed functional and structural connections. However, we believe that this alternative explanation is unlikely; in fact, our data also showed that functional connectivity reductions were associated with structural connectivity enhancements. In addition, the reduction of the DM-like subnetwork could be explained by indirect anatomical connections linked through a third-party region (Honey et al., 2009). Nevertheless, these 12 nodes within the DM-like subnetwork were one-to-one mirrored by the underlying impaired structural network. Numerous studies in healthy subjects have demonstrated that structural and functional resting-state connectivity are strongly interrelated, especially at the DMN level (Hagmann et al., 2008; Greicius et al., 2009; Honey et al., 2009; van den Heuvel et al., 2009a). Collectively, decreased functional connectivity within the DMN coupled with increased structural connectivity between highly central hubs might be used as a double biomarker in acute mTBI.

## Recovery of functional and structural connectivity

Overall, the group x time interaction resulted in a normalization of the connections of the patients over the year, characterized by an increase in functional connectivity and a decrease in structural connectivity (as well as in mean FA). Nevertheless, the compensatory reorganization of both functional and structural subnetworks was not the same for the different interaction approaches. The selective interaction that tracked the impaired functional and structural subnetworks found at Visit 1 reached only a partial normalization, in fact, differences between the groups weakened over time, but did not completely disappear until Visit 2. In contrast, the whole-brain interaction revealed a stronger restoration, marked by pronounced increases in functional connectivity and pronounced decreases in structural connectivity of the patients involving numerous nodes and connections. We attributed these diverse longitudinal scenarios to the quite dissimilar connections analyzed. In fact, the selective interaction only accounted for connections damaged within the first 7 days, whereas the whole-brain interaction accounted for connections affected later in the course of mTBI. For example, only two functional connections were shared by the 15-edge and 59-edge subnetwork resulted from the selective and the whole-brain approach, respectively. The prominent whole-brain compensation might be attributable to the local recruitment of new structural connections. The evolution of mTBI after the acute stage may reflect a transfer from global to more local brain communication, since the brain probably starts to reroute information traffic to nodes at a lower order to alleviate the hub overload of the highest nodes in the hierarchy (Stam, 2014). As months pass by, this strong local reorganization might initiate the normalization, leading to a more efficient balance between local and global information flow. The use of two complementary interaction approaches showed that different brain regions need different times to recover. Highly interconnected hubs of the connectome seem to be the hardest to fully recover after mTBI. In the literature, connectivity studies with a follow-up of 1 year after mTBI are challenging to find. Nevertheless, our results are consistent with prior prospective studies that reported partial recovery in functional connectivity after 6 months (Bharath et al., 2015; Sours et al., 2015a) and in structural diffusion metrics (decrease in FA) after 3–5 months (Mayer et al., 2010a; Ling et al., 2012). Other studies failed to detect longitudinal changes in resting-state functional connectivity during a 4-month (Mayer et al., 2011) and a 6-month period (Sours et al., 2015b).

A critical distinction with previous studies that exclusively assessed symptomatic patients in the chronic stage is further worthwhile, although the findings in this subset of patients may not be generalized to the entire mTBI population. Using rsfMRI data, pronounced decrease in graph properties in frontal regions were found in mTBI patients with persistent post-

concussion syndrome at the late phase in comparison to patients without complaints (Messe et al., 2013). By means of DTI, there is a growing consensus that FA is decreased in the chronic phase of symptomatic mTBI, especially in the corpus callosum, fornix, anterior corona radiata, uncinate fasciculus, inferior longitudinal fasciculus, and in the cingulum (Niogi et al., 2008; Niogi and Mukherjee, 2010; Eierud et al., 2014; Dean et al., 2015). Increased mean diffusivity in similar long association tracts as reported above has also been found in relation to poor outcome showing that those patients had greater and wider structural damages at late phase than patients with good outcome (Messe et al., 2011; Messe et al., 2012).

Although the duration of a full recovery is not yet clear, the current study suggests that neuroplasticity after mTBI necessitates more than 1 year to completely restore, both for structural and functional networks. As already reported in other studies, our findings are in agreement that residue of physiological anomalies are difficult to detect with standard clinical and cognitive assessments, as these symptoms were mostly already returned to baseline (Zhu et al., 2015). Finally, the degree to which network recovery differs between patients with and without persistent symptoms is under debate. At the functional level, the comparison between patients with and without PCD revealed no differences at Visit 2, suggesting decoupling between compensatory brain response to mTBI and clinical symptomatology. The structural recovery was analogous for symptomatic and asymptomatic patients when looking at the whole-brain recovery but indicated a slight trend toward distinctive courses when investigating the initially impaired subnetwork longitudinally. However, here only a handful of patients (n = 6) demonstrated PCD after 1 year and therefore a larger sample size is required to verify our findings. Other factors indirectly related to mTBI such as insomnia, fatigue, post-traumatic headache, pre-injury problems, or psychological distress might play a role in the maintenance of protracted symptoms after mTBI (Zumstein et al., 2011; Silverberg et al., 2015).

## Relationship between network and cognition over time

Some mTBI studies have supported the relationship between an anomal pattern of connectivity and poorer cognitive performance, especially in attention, executive function and working memory (Mayer et al., 2015a; Dall'Acqua et al., 2016). Only few studies looked at the correlation between changes in the brain and changes in cognition over a long-time period (Croall et al., 2014). Above, we showed a longitudinal increase in functional connectivity within the DM-like subnetwork in patients interpreted as reflecting recovery, which therefore may be linked to cognitive improvement. Unexpectedly, we found that the lower the functional connectivity of the DM-like subnetwork the better the cognitive performance in a working

memory and divided attention task. This correlation should be viewed in the context of the ability of the "recovered" DMN to attenuate its activity during goal-focused tasks (Greicius et al., 2003). The DMN is a network of functionally connected structures synchronously activated at rest and during internally directed processes, but synchronously deactivated during external task conditions (Raichle et al., 2001). The DMN has an essential role in cognitive functions and task-evoked activity is intimately related to functional connectivity identified in the resting brain (Smith et al., 2009; Laird et al., 2011). Failure to deactivate the DMN, in particular the PCC as one of its core nodes, has been associated with poor cognitive performance in mTBI and TBI, but also in healthy subjects (Weissman et al., 2006; Leech and Sharp, 2014; van der Horn et al., 2015b). Attenuation of DMN activity in order to allow the task-positive networks to be activated has been described as load-dependent: the greater the cognitive effort required from the task the stronger the inhibition of the DMN (Mayer et al., 2010b). In line with these observations, we hypothesized that patients able to robustly inhibit DMN activity performed better in cognitively high demanding tasks such as working memory and divided attention. This interpretation has not been explicitly tested in our study because our patients did not actively participate in an fMRI experiment that would allow a direct comparison between their brain activation and their behavioral responses.

Finally, the finding that decreased structural connectivity was related to verbal memory and divided attention worsening over time was puzzling due to its apparent contradiction. While speculative, we interpreted the decrease in structural connectivity (measured by both the number of streamlines as well as FA) as recovery mechanism since the majority of the patients recovered over time. On the contrary, the literature indicated that low FA findings are more frequently reported in association with poor neuropsychological performance in studies of chronic mTBI (Eierud et al., 2014). Nevertheless, the link between clinical outcomes and the direction of the FA change remain controversial and the authors of the meta-analysis also suggested that factors contributing to increased FA in the acute phase, i.e. increased intracellular and decreased extracellular water within the myelin sheath, are no longer valid in the chronic phase.

This idea is in line with our findings, since the longitudinal decrease in the number of streamlines over the year corresponded to a decrease in FA. After 1 year, the premorbid status, in which high structural connectivity leads to better performance, was restored again (Tamnes et al., 2011). Interpretations of the abovementioned correlations have to be done with caution since they were uncorrected for multiple testing.

## Limitations and conclusions

The present study has some limitations that are worth mentioning. To track the recovery until exhaustion of its potential, future longitudinal studies should scan beyond 12 months. The small number of mTBI patients (n = 6) having a PCD in the chronic phase (~12%) may affect robust interpretations and compromises the overall generalizability of the results. Even though this percentage is consistent with the mTBI literature (Hou et al., 2012), a larger sample size with symptomatic patients is required to improve the power of the statistical analysis. In addition, it should be considered that the patient group consisted of a convenience sample recruited from four different emergency department according to the availability of the study team and may not be representative of the whole mTBI population. At the same time, the study took advantage of the low long-term dropout rate, motivated through various incentives including financial compensation, free transport service and intensive contact strategy.

Finally, functional and structural alterations of the healthy subjects over time were somewhat unexpected and have the potential to complicate our understanding of the group x time interaction. Studies examining the long-term test-retest reliability of graph metrics derived from rsfMRI data have documented heterogeneous results, spanning from low to high network reliability in young adults (Wang et al., 2011; Franco et al., 2013; Du et al., 2015). These results are likely to be dependent on multiple factors such as the network identification analysis (whole-brain, seed-based or independent-component analysis) and the definition of long-term reliability. Even so, other evidence suggests that dynamic changes in resting-state networks might emerge depending on the subjective mental state of participants during scanning (Waites et al., 2005; Newton et al., 2007). A study using a mood-induction paradigm found increased functional connectivity with increasing subjective experience of sadness in a paralimbic network (Harrison et al., 2008). These findings fit well with the functional hyperconnectivity observed in the DM-like subnetwork of our controls at Visit 1 involving bilateral ACC and SMA. Collectively, low test-retest reliability in our controls might originate from the emotional state related to the first scan (e.g., anxiety, tension) that was no longer present at the second scan. In contrast, excessive fatigue and drowsiness of the patients due to their recent mTBI may have influenced their dominant subjective state at Visit 1, rather than any emotional involvement. Finally, change in functional connectivity within the healthy controls has been previously described in a TBI study (Hillary et al., 2011). Here – as in our study – the controls showed a decrease in functional connectivity, whereas the patients showed an increase between time points (3 months interval).

Studies on long-term test-retest reliability of network measurements derived from DTI

data throughout adulthood are still lacking. However, a study examining diffusion MRI during late adolescence over a 2-year period suggested increased structural connectivity between frontal and subcortical hubs (Baker et al., 2015). These authors reported evidence of selective refinement of connections in healthy adolescents over time and this maturational process might support the increase in structural connectivity over the year in our control sample. Similar observations were made in a recent DTI study in schizophrenia that employed graph theoretical analysis over a 5-year period and found an increase of nodal efficiency (global integration) in the healthy controls suggesting maturational and/or plasticity-related processes in the network as well as in a cross sectional study examining the structural connectome of healthy individual between 12 and 30 years that exhibited increased network integration (Dennis et al., 2013a; Sun et al., 2016). Lastly, confounding sources not completely controllable affecting intrasession DTI variability include subject head motion (Jones and Cercignani, 2010; Wang et al., 2012; Jones et al., 2013). We therefore investigated levels of head motion in our healthy controls between visits but did not find any significant translational or rotational head motions changes (average volume-by-volume translations $p = 0.618$ and average volume-by-volume rotations $p = 0.965$). We could so ensure that translational and rotational motions were comparable between Visit 1 and 2.

Our DTI analysis is based on standard procedures widely used in clinical settings that, however, have also their shortcomings. Although the single tensor model cannot deal with voxels containing crossing fibers, this limitation is present in both of our groups at both time points and hence should not bias the group comparisons and interactions reported. We recognize that there are fundamental limitations on the ability of tensor-based tractography to accurately estimate complex fiber configurations and that the use of higher-order tractography models on diffusion-weighted data such as constrained spherical deconvolution or automatic relevance determination would be definitively more favorable (Behrens et al., 2007; Tournier et al., 2007). These more sophisticated reconstruction algorithms clearly demonstrated fiber tracts more accurately in the presence of multiple fiber populations within a voxel (Farquharson et al., 2013; Jeurissen et al., 2013). Therefore, the results derived from DTI need to be interpreted with caution. For future diffusion imaging projects, we considered to apply superior diffusion models for the downstream processing as well as newer analysis tools such as MRtrix3.

Finally, linear “eddy_correct” to correct for eddy current-induced off-resonance field is widely used in clinical settings. Although we found equivalent effect on the structural connectome across the different corrections (eddy and eddy_correct), recent publications

revealed that this model is insufficient for diffusion data measured with high-b-values and that a higher order model performs better (Yamada et al., 2014; Andersson and Sotiropoulos, 2016). Future diffusion imaging studies should employ the eddy and topup tools, which offer superior correction for distortion and will provide more accurate insights into the structural network alterations following mTBI.

In conclusion, the current study demonstrates the involvement of networks similar to the DMN and of central hub areas in the pathophysiology of mTBI. Functional and structural compensatory processes differ between brain regions with respect to their time course and are not completed after 1 year, in particular when central hubs are involved. It remains unknown if the impaired functional and structural network connectivity will ever reach the premorbid level. This multimodal study highlights for the first time the importance of scanning the brain over a longer period than 1-year post-injury. Future longitudinal investigations should extend the time horizon to track down the full dynamics of neuronal plasticity, which could be used by clinicians to update their management, intervention and prevention after a single or repeated mTBI.

## 4.2 STUDY II

### Prefrontal cortical thickening after mild traumatic brain injury: a one-year magnetic resonance imaging study

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### 4.2.1 ABSTRACT

The objective of this study was to evaluate group-by-time interactions between gray matter morphology of healthy controls and that of patients with mild traumatic brain injury (mTBI) as they transitioned from acute to chronic stages, and to relate these findings to long-term cognitive alterations to identify distinct recovery trajectories between good outcome (GO) and poor outcome (PO). High-resolution T1-weighted magnetic resonance images were acquired in 49 mTBI patients within 7 days and 1-year post-injury and at equivalent times in 49 healthy controls. Using linear mixed-effects models, we performed mass-univariate analyses and associated the results of the interaction with changes in cognitive performance. Morphological alterations indexed by increased or decreased cortical thickness have been expected mainly in frontal, parietal, and temporal brain regions. A significant interaction was found in cortical thickness, spatially restricted to bilateral structures of the prefrontal cortex, showing thickening in mTBI and normal developmental thinning in controls. A discrete thickness increase that can interpreted as the absence of cortical thinning typically seen in the healthy population was associated with cognitive recovery in the GO subgroup, while the exaggerated cortical thickening in the PO patients was linked to worsening cognitive performance. Thickness of the prefrontal cortex is subject to structural alterations during the first year after mTBI. Beside beneficial neuroplasticity, a prolonged state of neuroinflammation for symptomatic patients (maladaptive neuroplasticity) cannot be excluded. If the underlying cellular processes responsible for cortical thickening following mTBI have been determined, brain stimulation or even pharmacological intervention targeting the prefrontal cortex might promote endogenous neural restoration.

### 4.2.2 INTRODUCTION

More knowledge about long-term spontaneous neuroplasticity is of great importance for improving prognosis and for optimizing therapeutic strategies after mild traumatic brain injury (mTBI). This is particularly true for a considerable minority of patients who continue to experience post-concussion impairments at least 1 year after trauma and thus are at risk for adverse functional outcomes (Sterr et al., 2006). Sometimes the chronic consequences are severe enough to impair their quality of life and to interfere with personal, occupational, and social functioning. Further, the high prevalence and incidence of mTBI lead to considerable economic and social burdens (Levin and Diaz-Arrastia, 2015). Standard clinical brain scans such as those from computed tomography (CT), which typically appears normal and have low spatial resolution, are not capable of detecting and monitoring adaptive cortical remodeling.

In recent years, longitudinal changes in morphology following mTBI have been increasingly investigated. Studies focusing on patients with chronic post-traumatic symptoms or with repetitive concussive episodes have reported decreased gray matter volume or cortical thickness (Ross et al., 2012; Zhou et al., 2013; Tate et al., 2014; Meier et al., 2015). Cortical volume reduction also has been observed in typical mTBI patients over 1 month after injury (Toth et al., 2013), but investigations over a 3-month interval have identified both cortical thinning and cortical thickening (Wang et al., 2014; Govindarajan et al., 2016). One prospective study examining gray matter atrophy yielded neither cortical nor subcortical differences between patients and matched controls at 4-month follow-up (Ling et al., 2013). The inconsistency of these findings might arise from differing methodologies, diverse statistical approaches, and the time interval post-injury. Long-term spontaneous neuroplasticity and outcome-specific evolution after mTBI still remain poorly understood and these morphological brain alterations remain even more demanding to quantify and monitor.

Here, we investigated gray matter biomarkers in 49 mTBI patients and 49 healthy controls as they passed from an acute (< 7 days) to a more chronic stage (approximately 1 year) and correlated these morphological brain alterations with changes in cognitive functioning. Finally, we sought to differentiate recovery- and reorganization-related cortical dynamics associated with good outcome (GO) and poor outcome (PO) after mTBI. Neuromorphological alterations in the form of increased or decreased cortical thickness, surface area, or volume have been expected mainly in frontal, parietal, and temporal brain regions. With the scarcity of mTBI studies in the adult population focusing on surface-based morphometric measures such as cortical thickness and with the inconsistent results reported in the few existing studies – spanning from cortical thinning to cortical thickening – we preferred

to avoid the formulation of a more detailed hypothesis with respect to the direction of the alterations (decreases or increases).

### 4.2.3 MATERIALS AND METHODS

#### Subjects

Measurements of 49 patients with acute mTBI (mean age 34.9 ± 12.4, range 18–61 years) and 49 sex-, age-, and education-matched controls (mean age 35.0 ± 12.1, range 18–60 years) were investigated. Based on EFNS guidelines, participants were enrolled in four local hospitals from February 2012 to March 2014 and follow-up continued until March 2015. Inclusion criteria included age 18 to 64 years, normal CT, and a clear mTBI following the EFNS criteria (initial GCS score of 13–15; loss of consciousness <30 min or qualitative alteration in mental status and/or presence of post-traumatic amnesia <60 min and/or retrograde amnesia <30 min). Exclusion criteria for all participants comprised a history of pre-existing neurologic/psychiatric disorders, prior TBI, attention deficit/hyperactivity disorder, alcohol or substance abuse, and any MRI incompatibility. In addition, subjects with intracranial pathology on conventional MRI scans were not included in the study.

The patient cohort underwent neuroimaging and clinical evaluations within 2 to 7 days after injury (Visit 1) and 1 year later (Visit 2). The controls underwent similar scans at the same time interval. The initial study population was composed of 51 mTBI patients and 53 controls at Visit 1 (already excluding two patients and one healthy control because of incidental brain anomalies); after 1 year, two patients did not return for the second visit due to pregnancy and emigration. Finally, to guarantee an equal number of subjects in each group, the control group also was reduced to 49 participants, ensuring a 1:1 matching of key demographic characteristics.

#### Standard protocol approvals, registrations, and patient consents

Two Swiss Cantonal Ethics Committees approved the study. The study was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent prior to study enrollment.

#### Neuropsychological assessment

A comprehensive neuropsychological and clinical examination was administered to all subjects at each visit (descriptions are provided in the Supplementary Material B.1). Tests covered attention/executive functioning (alertness, Go-Nogo, divided attention), memory

(working memory, and verbal memory composite consisted of learning and delayed recall), and measure of effort (malingering). Depressed mood, anxiety, and post-concussive symptoms also were evaluated. Post-injury outcome was assessed using the RPQ, which lists 16 commonly experienced complaints on a scale from 0-1 (symptom-free) to 4 (severe) (King et al., 1995). The RPQ was used to dichotomize the patients into a PO subgroup reporting at least three symptoms of moderate-to-severe level and a GO subgroup having minimal or no symptoms. To be assigned to the PO subgroup, patients had to fulfill the PO criteria at Visits 1 and 2.

## Imaging protocol and preprocessing

MRI scans were acquired on a 3.0 Tesla Philips Ingenia whole–body scanner (Philips Medical Systems, Best, the Netherlands) equipped with a transmit-receive body coil and a commercial 15-elements transmit-receive head coil array that is capable of sensitivity encoding (SENSE). A volumetric 3D T1-weighted gradient echo sequence (turbo field echo) image was measured with a spatial resolution of 1.0 x 1.0 x 1.0 $mm^3$ (acquisition matrix 240 x 240 pixels, 160 sagittal slices) and reconstructed to a spatial resolution of 0.94 x 0.94 x 1.0 $mm^3$ (reconstruction matrix 256 x 256 pixels, 160 sagittal slices). Further imaging parameters were: field of view = 240 x 240 $mm^2$, echo time = 3.70 msec, repetition time = 8.14 msec, flip-angle $\alpha = 8°$, SENSE factor R = 1.8, acquisition time (min) 7:29. Clinical MRI scans such as T2-weighted, proton-density-weighted, susceptibility weighted imaging and fluid attenuated inversion recovery were additionally acquired and evaluated by the same radiologist in order to exclude intracranial pathology (defined as exclusion criteria).

Cortical surface reconstruction was performed with the FreeSurfer image analysis suite (version 5.3.0), which is documented and freely available online (http://surfer.nmr.mgh.harvard.edu). The technical details of these procedures are described in prior publications (Dale et al., 1999; Fischl et al., 1999a; Fischl et al., 1999b; Fischl and Dale, 2000; Fischl et al., 2001; Fischl et al., 2002; Fischl et al., 2004a; Fischl et al., 2004b).

The 3D structural high resolution T1-weighted MRI scan was used to construct models of each subject's cortical surface in order to measure cortical thickness and surface area. This fully automated procedure comprised segmentation of the cortical and subcortical white matter (Dale et al., 1999), tessellation of the gray matter/white matter boundary, inflation of the folded surface tessellation patterns (Fischl et al., 1999a; Fischl et al., 1999b) and automatic correction of topological defects in the resulting manifold (Fischl et al., 2001). This cortical surface was then used as starting point for a deformable surface algorithm designed to find the gray/white and pial (gray matter/cerebrospinal fluid) interfaces with sub-millimeter precision (Fischl and

Dale, 2000). The procedures for measuring cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003; Salat et al., 2004). This method uses both intensity and continuity information from the surfaces in the deformation procedure in order to interpolate surface locations for regions in which the MRI scan is ambiguous (Fischl and Dale, 2000).

For each subject, cortical surface area, thickness, and volume of the cortical ribbon was computed on a uniform grid (comprised by vertices) with about 1 mm spacing across both cortical hemispheres, with the thickness being defined by the shortest distance between the gray/white and pial surface models. The thickness maps produced are not limited to the voxel resolution of the image and thus sensitive for submillimeter differences between groups (Fischl and Dale, 2000). The way in which the resolution of the cortical thickness maps goes beyond the resolution of the original acquisition is conceptually similar to a (conventional) partial volume correction procedure. The cortex is smooth at the spatial scale of a several millimeters, which is imposed as constraint by FreeSurfer to estimate the location of the surface with subvoxel accuracy. For instance, if a given voxel is darker than its neighboring gray matter, it probably contains more cerebrospinal fluid and therefore the surface model is at a slightly different position than if the neighboring voxels were brighter and therefore contain probably more white matter. Cortical surface area, thickness, and volume measures were mapped onto the inflated surface of each participant's brain reconstruction, thus allowing visualization of data across the entire cortical surface (gyri and sulci) without the data being obscured by cortical folding. Data were re-sampled for all subjects and rendered onto a common spherical coordinate system (Fischl et al., 1999b).

Then, surface-based, vertex-wise cortical surface area, thickness, and volume maps were computed for each participant. For the whole–brain vertex-wise analysis, the data were smoothed on the surface tessellation using an iterative nearest-neighbor averaging procedure with 166 iterations on the left hemisphere and 167 iterations on the right hemisphere, corresponding to a two-dimensional surface-based diffusion-smoothing kernel with a full width at half maximum of about 15 mm. These cortical surface area, thickness, and volume maps were then subjected to statistical analyses using statistical tools implemented in FreeSurfer. We used standard nomenclature as implemented in FreeSurfer to label the anatomical structures (Desikan et al., 2006). Quality assessment was done using FreeSurfer's quality assessment tools (https://surfer.nmr.mgh.harvard.edu/fswiki/QATools) and no manual interventions were necessary. The longitudinal automated pipeline was run to obtain cortical volume, thickness, and surface area changes between the scans (Reuter et al., 2012). Based on the robust inverse

consistent registration method, within-subject templates for each subject unbiased towards any time-point were created (Reuter et al., 2010). After that, different processing steps, including skull stripping, Talairach transformations, atlas registration, subcortical segmentation, spherical surface maps, and parcellations, were initialized within common information from the within-subject template to improve reliability and statistical power (Reuter et al., 2012).

### Statistical analysis

To determine vertex-wise changes in cortical morphometry over the year, mass-univariate image analyses were performed using a linear mixed-effects (LME) model implemented as MATLAB scripts distributed with FreeSurfer (Bernal-Rusiel et al., 2013). In the model, fixed effects included group (patient, control), time (Visit 1, Visit 2), and the interaction term between group and time; the intercept was included as random effect. The export of the imaging results of each cluster to IBM SPSS 23.0 enabled a closer evaluation of the main effects of the interaction (averaged across clusters), of PO and GO subgroup effects, and of partial Spearman correlations between morphological changes and changes in cognitive functioning (difference between Visits 1 and 2) over the year. Since the correlations were performed only in the patient group, we controlled for age, education, and total gray matter volume. In addition, since the PO and the GO subgroups trend-wise differed in the years of education, we corrected for education when directly comparing the two clinical subgroups.

Next, group comparisons at Visit 1 and 2 also were examined in FreeSurfer using whole-brain vertex-wise general linear models. In addition, group differences at Visit 2 also were conducted with a region-of-interest approach, namely within the clusters identified by the LME model as differentially altered over time (interaction analysis). Monte Carlo simulation was applied to control for multiple comparisons (5000 permutations), and the cluster extent threshold was set at $p < 0.05$. Although for a nominal family-wise error rate of 5%, the parametric statistical methods are shown to be invalid for cluster-wise inference. The nonparametric permutation test, such as the Monte Carlo simulation approach used in the current study, is found to produce nominal results for cluster-wise inference while offering precise control of false positives (Eklund et al., 2016). Results are reported two-sided (significance level at 0.05).

Analyses of demographic and clinical data between and within groups were conducted using t-tests for independent and dependent samples and using analysis of variance and covariance (repeated measures analysis of variance and analysis of covariance, respectively) when comparing the PO and GO subgroups.

### 4.2.4 RESULTS

#### Demographic and neuropsychological characteristics

Groups were matched for demographic characteristics (Table 4.2.1). Most patients had a GCS of 15 (n = 40), except for eight patients with a GCS of 14 and one patient with a GCS of 13. Causes of injury were sport accidents (23%), cycling accidents (23%), falls (23%), motor vehicle accidents (11%), and others (20%). Based on a semi-structured interview, neither the patients nor the healthy controls were taking drugs during the data collection, except for few patients taking analgesics at Visit 1. Patients underwent the first scan on average 4.9 ± 1.47 days post-injury; inter-scan interval was 365.9 ± 4.0 days for patients and 364.6 ± 5.1 days for controls ($p = 0.18$).

Patients showed lower cognitive performance across multiple neuropsychological tests – that is, in the domain of attention (alertness, selective attention/inhibitory control, divided attention) and working memory – and significantly more severe post-concussive symptoms on the RPQ than controls at Visit 1 (uncorrected for multiple comparisons). The majority of cognitive and clinical scores improved significantly over time, revealing no differences between groups except for divided attention. Nevertheless, persistent complaints still were significantly higher in patients after 1 year (Table 4.2.1). Six PO and 43 GO patients were identified according to RPQ at both visits. The patient subgroups did not differ on key measures (age, sex, GCS), but some differences were observed in years of education ($df = 47$, $t = 1.895$, $p = 0.06$), indicating that GO patients had more years of schooling (12.84 ± 2.5 years) than the PO subgroup (10.83 ± 1.5 years). We tested post hoc whether there is a relationship between GCS and the total score of the RPQ at Visit 1 or 2. Neither the total RPQ score at Visit 1 nor the one at Visit 2 were statistically significantly correlated with the GCS, by means of a partial correlation using education, age, and total gray matter volume as covariates of no interest.

**Table 4.2.1 Demographic and clinical characteristics**

| | Visit 1 | | Visit 2 | | Group effect Visit 1 | Group effect Visit 2 | Time effect (Patients) |
|---|---|---|---|---|---|---|---|
| | mTBI mean (SD) | HC mean (SD) | mTBI mean (SD) | HC mean (SD) | p-value | p-value | p-value |
| **Demographics** | | | | | | | |
| Age (y) | 34.9 (12.4) | 35.0 (12.1) | 35.9 (12.4) | 36.0 (12.1) | 0.96 | 0.96 | NA |
| Sex (male / female) | 18 / 31 | 18 / 31 | 18 / 31 | 18 / 31 | 1 | 1 | NA |
| Education (y) | 12.6 (2.5) | 12.9 (2.4) | 12.7 (2.5) | 13 (2.5) | 0.49 | 0.44 | 0.02 |
| **Neuropsychological assessment** | | | | | | | |
| Alertness (RT) | 243.9 (57.3) | 220.9 (20.3) | 217.6 (18.8) | 216.5 (19.9) | 0.01 | 0.79 | 0.002* |
| Go/Nogo (RT) | 398.3 (69.1) | 370.1 (45.2) | 380.4 (62.2) | 359.2 (46.1) | 0.02 | 0.06 | 0.02* |
| Divided attention (RT) | 695.5 (78.8) | 658.3 (69.7) | 647.7 (65.0) | 619.4 (66.5) | 0.01 | 0.03 | <0.001* |
| Working memory (score) | 5.4 (1.2) | 5.8 (1.3) | 5.9 (1.4) | 6 (1.2) | 0.05 | 0.64 | 0.001* |
| Verbal memory | -0.38 (0.9) | -0.16 (0.9) | 0.19 (0.8) | 0.35 (0.7) | 0.27 | 0.34 | <0.001* |
| RPQ (score) | 14.2 (10.8) | 2.8 (3.9) | 7 (9.9) | 2.5 (4.4) | <0.001 | 0.005 | <0.001 |
| BDI-II (score) | 6.4 (5.8) | 3.5 (4.4) | 4.1 (4.8) | 2.8 (4.3) | 0.01 | 0.17 | 0.003 |
| BAI (score) | 2.8 (6.6) | 0.6 (2.2) | 1.9 (4.5) | 0.14 (1.0) | 0.03 | 0.01 | 0.4 |
| MSVT | - | - | Good effort | Good effort | | | |

*All p values survived the adjustment for multiple testing (false discovery rate <0.05).

Alertness (mean of tonic and phasic alertness), divided attention (mean of visual and auditory scores), and verbal memory (mean z-score of learning and long delay from the German adaptation of the Rey Auditory Verbal Learning Test) represent composites.

Abbreviations: mTBI, mild traumatic brain injury; SD, standard deviation; HC, healthy controls; NA, not applicable; RT, reaction time in msec; RPQ, Rivermead Post-Concussion Symptoms Questionnaire; BDI-II, Beck Depression Inventory, 2nd edition; BAI, BeckAnxiety Inventory; MSVT, Medical Symptom Validity Test.

### Whole-brain group-by-time interaction in cortical morphology (FreeSurfer)

A significant group-by-time interaction computed vertex-wise in FreeSurfer was observed bilaterally in frontal regions showing cortical thickening in patients and cortical thinning in controls (cluster-wise p value corrected for multiple comparisons p = 0.0002 for both frontal clusters; Fig. 4.2.1).

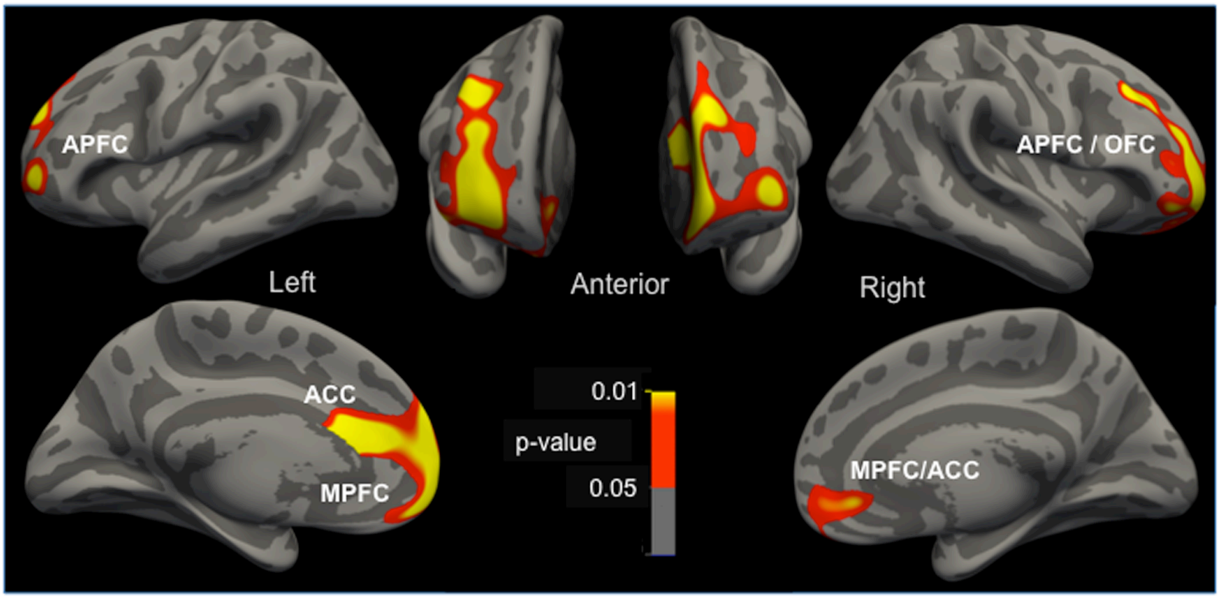


**Figure 4.2.1. Longitudinal alterations in cortical thickness**

The whole-brain group-by-time interaction exhibited significant cortical thickening in frontal regions in the mTBI patients (red-yellow). No regions showed cortical thinning in patients. Abbreviations: APFC, anterior prefrontal cortex; OFC, orbitofrontal cortex; MPFC, medial prefrontal cortex; ACC, anterior cingulate cortex.

These frontal clusters – referred from here as the PFC – involved the anterior prefrontal cortex (APFC), orbitofrontal cortex (OFC) and the medial prefrontal cortex (MPFC) extending into the ACC (Table 4.2.2).

**Table 4.2.2 Cortical thickness alterations over 1 year (group-by-time interaction) and differences at Visit 2 (group comparison, region of interest approach)**

| Measure and anatomical location (cortical thickness) | Cluster size ($mm^2$) | MNI coordinates | | | CWP |
|---|---|---|---|---|---|
| | | x | y | z | |
| **Whole-brain group-by-time interaction: 49 patients (PO & GO) and 49 controls** | | | | | |
| Left cluster: APFC, MPFC, ACC | 4,255.1 | -5.2 | 29.8 | 17 | 0.0002 |
| Right cluster: APFC, OFC, MPFC, ACC | 5,243.3 | 27.6 | 48.9 | -2.6 | 0.0002 |
| **Whole-brain group-by-time interaction: 43 patients (GO) and 49 controls** | | | | | |
| Left cluster: APFC, MPFC, ACC | 2,265.3 | -5.2 | 30.8 | 16.8 | 0.015 |
| Right cluster: APFC, OFC | 3,450.8 | 27.0 | 49.8 | -3.9 | 0.0004 |
| **Group comparison at Visit 2 (region-of-interest approach)** | | | | | |
| Left cluster: APFC, MPFC | 2,186.8 | -31.1 | 49.5 | 2 | 0.0002 |
| Right cluster: APFC | 1,396.8 | 31.2 | 49.6 | 3.9 | 0.0002 |
| Right cluster: DLPFC | 379.4 | 34.7 | 27.7 | 40.4 | 0.027 |

Abbreviations: MNI coordinates, coordinates of the maximum value found in the cluster within the MNI space; CWP, cluster-wise corrected p value; PO, poor outcome; GO, good outcome; APFC, anterior prefrontal cortex; MPFC, medial prefrontal cortex; ACC, anterior cingulate cortex; OFC, orbitofrontal cortex.

Changes in cortical thickness were not statistically significantly correlated with age or education, neither across both groups nor within the patient or control group separately. We checked for outliers in the patient group as well as in the PO and GO subgroup and did not find any outlier with respect to the cortical thickness alterations. The group-by-time interactions revealed no statistically significant findings for cortical surface area or cortical volume.

We did not explore the main effect of group (independent of time) and the main effect of time (independent of group) within the vertex-wise LME model in FreeSurfer. Instead we exported the mean (across vertices) thickness values of each statistically significant cluster obtained with the group-by-time interaction to SPSS to visualize cortical thickness trajectories and for further statistical analyses (time and group effect; see below).

## Time effects in cortical morphology within the prefrontal clusters (SPSS)

Within-group longitudinal analyses of cortical thickening in mTBI showed only a statistical trend when considering both prefrontal clusters together ($p = 0.085$), while controls showed significant ($p < 0.001$) cortical thinning typical of young adults across the span of 1 year (Fig. 4.2.2A).

However, when considering cluster-specific time effects in cortical thickening in mTBI patients, thickness increases were statistically significant in the right PFC cluster (p = 0.027), but not in the left PFC cluster (p = 0.40).

The rate of thickness change over the 12 months within the prefrontal regions (averaged over all vertices within the clusters) was +0.8% for the patients and -2.0% for the controls (Fig. 4.2.2A). However, it is important to note that the percentage of relative change in the patients, compared with the typical trajectory shown by the controls, amounted to 2.8%. Patients with PO showed greater thickening (time effect: annual change, +2.3%; p = 0.066) than patients with GO (time effect: annual change, +0.6%; p = 0.24).

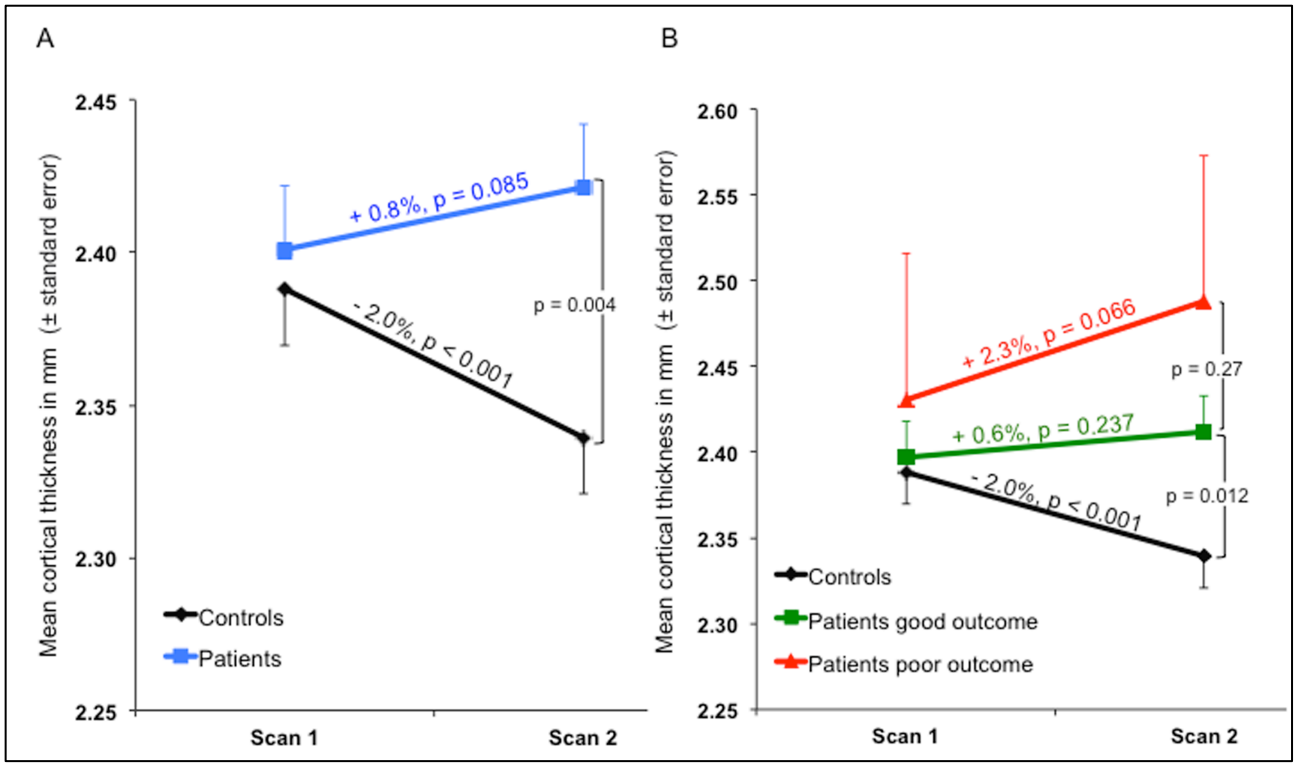


**Figure 4.2.2. Trajectories of cortical thickness**

Graph plots depict the trajectories of cortical thickness in prefrontal regions (average of left and right prefrontal clusters) for groups from Visit 1 to Visit 2. Error bars represent the standard error of the mean.

Note that the time effect in the GO subgroup was not statistically significant, whereas the time effect of the PO subgroup showed only a trend towards significance (Fig. 4.2.2A). Taking into account the annual longitudinal thinning of the PFC in the control group, the magnitude of averaged annualized thickening was 4.3% in the PO subgroup and 2.6% in the GO subgroup. Probably due to small sample size of the PO subgroup, the mean trajectory of this clinical subgroup between Visits 1 and 2 was not statistically significantly different (subgroup-by-time

interaction, p = 0.43; Fig. 4.2.2B). The cortical thickness trajectory for each individual subject separated by group is illustrated in Supplementary Figure B.1. In mTBI patients, the rate of annual thickening (averaged over all vertices within each cluster) was greater in the right PFC (+1.3%; p = 0.027) than in the left PFC (+0.4%; p = 0.39).

### Group effects in cortical morphology within the prefrontal clusters (SPSS)

At Visit 1, there were no statistically significant differences with respect to cortical thickness, neither between mTBI patients (PO and GO group pooled) and controls (Fig. 4.2.2A), nor between the controls and the GO subgroup and the controls and the PO subgroup, nor between the PO and GO subgroups (Fig. 4.2.2B). In contrast, mTBI patients (PO and GO group pooled) showed increased thickness, compared with controls at Visit 2 (p = 0.004; Fig. 4.2.2A). When comparing the patient subgroups separately with the controls, both subgroups showed increased thickness at Visit 2 (GO, p = 0.012; PO, p = 0.013), whereas PO patients did not differ from GO patients in cortical thickness at Visit 2 (p = 0.27; Fig. 4.2.2B).

### Group comparisons in the acute and chronic states of mTBI patients (FreeSurfer)

Based on cortical thickness averaged across clusters, we also reported vertex-wise group effects to obtain information whether there were already cortical thickness differences within the first week after mTBI (Visit 1) or still differences 1 year later (Visit 2) in prefrontal regions and beyond them. No differences in cortical thickness were identified between patients and controls in the acute phase (Visit 1).

One year later, however, mTBI patients, compared with controls, showed increased thickness within the prefrontal regions revealed by the interaction analysis (left cluster: p = 0.0002; right clusters: p[APFC] = 0.0002 and p[DLPFC] = 0.027, Fig. 4.2.3 and Table 4.2.2).

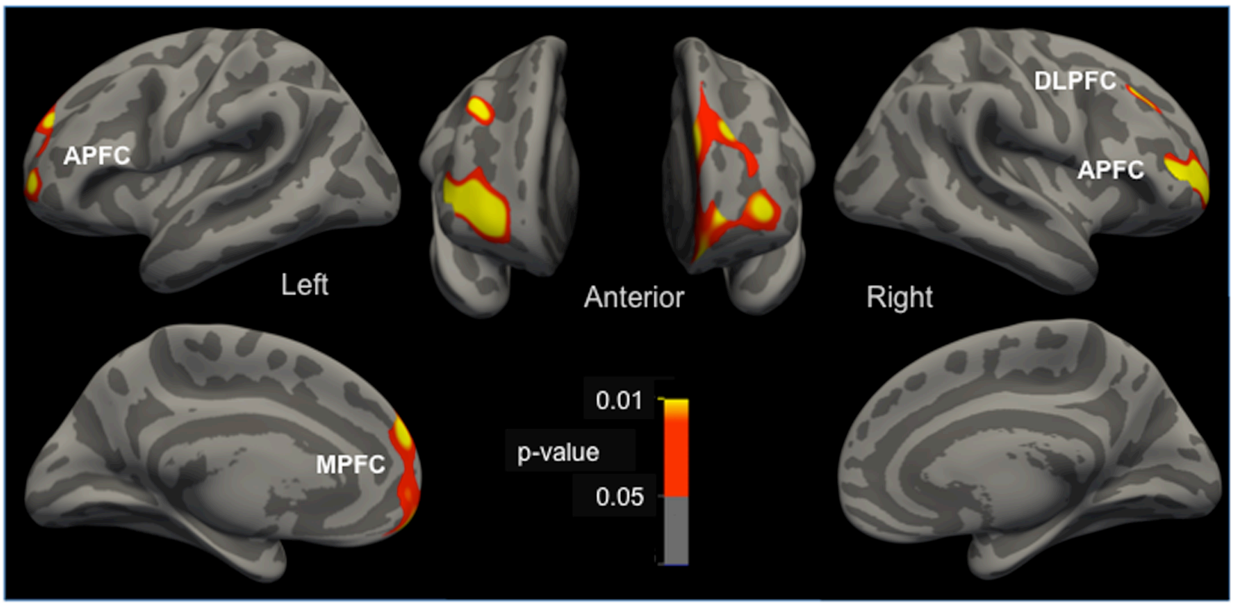


**Figure 4.2.3. Differences in cortical thickness of patients and controls in the chronic phase (Visit 2; region-of-interest approach)**

Cortical thickness is increased (red-yellow) in bilateral anterior prefrontal cortex (APFC), left medial prefrontal cortex (MPFC) and right dorsolateral prefrontal cortex (DLPFC) in mild traumatic brain injury patients, compared with controls at Visit 2.

Areas of increased prefrontal thickness were less extensive at 1-year post-injury than the clusters detected in the interaction analysis. Post hoc analysis revealed no differences between GO and PO patients when analyzing the average of the prefrontal clusters ($p = 0.15$). Increased thickness in the PO, compared with the GO subgroup, was observed in the right APFC cluster ($p = 0.045$), but not in the right DLPFC ($p = 0.34$) or left PFC cluster ($p = 0.30$).

Group comparison at Visit 2 using a whole-brain approach yielded similar results as those obtained with the cluster derived from the interaction, with an additional thickening in the superior and middle occipital gyrus as well as in the right intraparietal sulcus (Supplementary Fig. B.2 and Supplementary Table B.1). There were no significant group differences with respect to cortical surface area and volume at Visit 1 nor at Visit 2.

## Correlations between cortical thickness alterations and cognitive recovery (SPSS)

We found weak correlations between improvements in cognitive performance and prefrontal thickening over time in the mTBI group (Supplementary Table B.2). Positive correlations were observed between average prefrontal thickening and both working memory and verbal memory performance increases (rho = 0.236, $p = 0.057$ and rho = 0.302, $p = 0.021$, respectively; uncorrected). A negative correlation was observed between average prefrontal

thickening and changes in reaction time of the Go/Nogo task (rho = -0.236, p = 0.057; uncorrected). None of these results survived the post hoc adjustment with false discovery rate (FDR; < 0.05) for multiple comparisons. Similar correlations between improvements in cognitive performance and prefrontal thickening over time were analyzed separately within the patient subgroups. These yielded significant correlations in the GO subgroup (for Go/Nogo task, working memory, and verbal memory) and in the PO subgroup (for Go/Nogo and divided attention). However, these results also did not survive FDR correction. See Supplementary Table B.3 for FDR-adjusted values and Supplementary Figure B.3 for graph plots.

### 4.2.5 DISCUSSION

To the best of our knowledge, this is the first study that applied a longitudinal design with a relatively large sample of mTBI patients and healthy control subjects to investigate alterations in brain morphology over the first year after mTBI. We observed a significant group-by-time interaction in cortical thickness, mainly found in bilateral structures of the PFC (APFC, MPFC, OFC, and ACC). This interaction was qualified by a thickening of the cortex in mTBI patients (4.3% change in the PO subgroup and 2.6% change in the GO subgroup), compared with controls who exhibited the normal developmental trajectory characterized by cortical thinning (Wierenga et al., 2014; Amlien et al., 2016; Walhovd et al., 2016).

Group comparisons at 1-year post-injury thus uncovered increased prefrontal thickness in mTBI patients, compared with controls. This had not been evident in the acute phase. However, we previously reported a reduction in cortical surface area associated with stronger subjective complaints in bilateral PFC, OFC, and ACC in the same cohort within 7 days after mTBI (Dall'Acqua et al., 2016). The reason why reduced cortical surface area is associated with the symptomatology in these regions in the acute phase and cortical thickness increases in the chronic phase 1-year post-injury as revealed in the present study is not known, and we avoid speculating about it in the following discussion.

At present, a few longitudinal studies have indicated cortical thickness changes in a typical sample of single mTBI, and they have described thinning (Wang et al., 2014; Mayer et al., 2015b; Govindarajan et al., 2016; Urban et al., 2016), thickening (Wang et al., 2014; Govindarajan et al., 2016), and no alterations over time (Ling et al., 2013).

The mTBI literature based on children and adolescence consistently exhibited a decrease in cortical thickness, at least during the first few months post-injury. For example, a longitudinal MRI study with a pediatric sample displayed cortical thinning in the bilateral frontal gyri, as well as in the temporal, parietal and occipital cortex of the left hemisphere at 4

months post-injury (Mayer et al., 2015b). A recent cross-sectional study confirmed these results and detected a thinner cortex in frontal and parietal regions in asymptomatic children and adolescents who sustained a mTBI 3–4 months prior to participation, compared with typical developing controls (Urban et al., 2016). These findings seem to indicate that a mTBI disrupts the neural maturation of the developing brain by ‘‘synaptic overpruning’’ in cortical regions subjected, at the same time, to normal thinning elicited by pruning and apoptosis (Dennis et al., 2013b).

In the literature that investigated adults, mTBI is associated with the coexistence of both cortical thinning and thickening across multiple brain regions. For example, a group of motor vehicle collision survivors showed thinner cortex in the left posterior middle temporal gyrus, but thicker cortex in the right precuneus and in the left rostral middle frontal gyrus at 7 days post-mTBI (Wang et al., 2014). Only the last-mentioned region had a subsequent decrease at 3 months, although the mean thickness was still elevated, compared with that of the non-mTBI group. Another publication observed cortical thinning at around 24 h following mTBI in left temporal and, transiently, right parietal regions that altered into small-scattered spots of subtle cortical thickening and thinning in different regions of the left hemisphere at around 3 months post-injury (Govindarajan et al., 2016).

In the current study, the use of the more sensitive and specific LME model may have enhanced the detection of dynamic and subtle brain alterations after mTBI (Bernal-Rusiel et al., 2013). Further, these studies only considered the sub-acute phase of mTBI (< 4 months post-injury) and applied predominantly between- and within-group analyses rather than the more appropriate group-by-time interaction.

While no comparable longitudinal work is available for mTBI, our finding of cortical thickening is consistent with a recent study of a cross-sectional sample of sports-related mTBI assessed at 1, 3, 6, and 12 months post-injury (Killgore et al., 2016). This showed that longer recovery time was correlated with increased gray matter volume within the bilateral ventromedial PFC, which was in turn associated with improved cognitive and emotional functioning. The authors interpreted these findings as evidence that experience-dependent cortical plasticity, promoted through regular practice, may compensate for their deficits. This hypothesis appears plausible, since it is well documented that brain structures that undergo repeated training show compensatory remodeling via increased dendritic arborization and increases in spine density (Kerr et al., 2011; Kolb and Gibb, 2015).

Cortical thickening over a long-term interval also was observed in children who sustained moderate to severe TBI (Wilde et al., 2012). This study assessed longitudinal changes in

cortical thickness between 3 and 18 months post-injury and also found thickening in large bilateral medial frontal regions in TBI, compared with controls. Cortical thickness change within these regions was correlated with greater symptom severity in emotional control and behavioral regulation and was explained by a failure in neuronal pruning caused by the injury (maladaptation) (Wilde et al., 2012).

There is agreement that monotonic cortical thinning across the entire cortex, starting in early childhood (3 years or younger) and continuing into young adulthood (30 years and beyond), represents normal brain development in the healthy population (Wierenga et al., 2014; Amlien et al., 2016; Walhovd et al., 2016). Some have suggested that cortical thinning is important for efficient information processing in early adolescence through use-dependent synaptic pruning that selectively eliminates excess or underutilized synapses (Blakemore, 2012). Widespread thinning of the healthy cortex also has been reported across the adult life span (Fjell et al., 2009; Westlye et al., 2010; Storsve et al., 2014). The rate of thickness change varies across cortical regions but appears especially prominent in prefrontal cortices where annual reduction generally exceeds 1.5%, in accordance with the observation within our control group (Fjell et al., 2009; Tamnes et al., 2013). Prefrontal regions are not only the last to mature (i.e., last to prune), but are especially vulnerable to age-related atrophy and disease (Bullmore and Sporns, 2012; Tamnes et al., 2013; van den Heuvel and Sporns, 2013).

In the current study, mTBI seems to selectively affect prefrontal regions, which are known to house central nodes of different brain networks, irrespective of the heterogeneous locations of the biomechanical impact. The PFC in particular, sometimes called a “connector hub”, is a high-centrality node connecting to various brain modules in a network and is crucial for global integration and adaptive behavior (Bullmore and Sporns, 2012). This structural hub has a higher proportion of long-distance connections than less central nodes and has high metabolic demands. Moreover, prefrontal network nodes are more vulnerable to neuropathological attack, which can cause widespread damage to the network’s global efficiency (Bullmore and Sporns, 2012; van den Heuvel and Sporns, 2013). Since mTBI damages axonal fibers, it is plausible that the PFC is a hotspot of persistent cognitive and emotional symptomatology. Brain hubs are often more likely to be affected in diverse brain disorders (Crossley et al., 2014). A recent study has also proposed that brain disorders might be initiated in peripheral nodes and only become dysfunctional once these disorders have also affected topologically central nodes (Crossley et al., 2014).

In the GO subgroup, we found that thickness change in prefrontal clusters (+0.6%) was longitudinally associated with cognitive recovery (improved selective attention/executive

control, working memory, and verbal memory). This might be indicative of experience-dependent beneficial plasticity via modification of lifestyle in daily life (Kerr et al., 2011) or simply show the absence of the typical cortical thinning exhibited by the healthy controls. On the other hand, the strong thickening detected in the small minority of PO patients (+2.3%) also was associated with performance worsening in divided attention (see Supplementary Table B.3). Although this result is based on a small sample (n = 6), the substantial thickening in prefrontal areas may affect cognitive functioning adversely, which would support the idea that neuropathological underpinnings foster unfavorable outcome of mTBI. Nevertheless, divergence between the trajectories of the patient subgroups did not achieve significance. The reason for this may intuitively be attributed to the small sample size of the PO subgroup.

The process of cortical reorganization in patients seems to persist for at least 1 year after mTBI, later than the recovery of cognitive capacities. Further longitudinal research should examine whether this lack of maturational thinning is temporary or whether the cortical remodeling represents a stable compensatory mechanism.

Experiences constantly shape the nervous system structure and vice versa. Following a brain injury, behavioral experiences including self-taught compensatory strategies and enriched environment exposure, interact with the neural environment by synaptogenesis, dendritic growth, and gliogenesis, among other mechanisms to promote restorative neuroplasticity (Kerr et al., 2011; Zatorre et al., 2012). Neuroplasticity in the damaged brain can vary with training intensity, age, and timing (Kerr et al., 2011). However, neuroplasticity is not always beneficial. Beneficial neuroplasticity in mTBI patients might be driven by the absence of normal developmental cortical thinning as we observed mainly in the patients with GO and is correlated with improvements in the Go/Nogo task, working memory, and verbal memory. On the contrary, maladaptive neuroplasticity might be driven by neuroinflammation in addition to the absence of cortical thinning, resulting in exaggerated cortical thickening as we observed mainly in the patients with PO, and this thickening is correlated with worsening in divided attention. We do not have histological data, and extra caution is required when attempting to relate macroscopic changes in imaging biomarkers to biological tissue mechanisms.

Candidate processes for the long-term cortical thickening observed in the PO subgroup are based on animal models, human post mortem studies, and in vivo repetitive concussion, and include increased reactive proliferation of astrocyte and microglia in the chronic phase (Hernandez-Ontiveros et al., 2013; Smith et al., 2013; Pekny et al., 2014; Coughlin et al., 2017; Shahim et al., 2017). Astrocyte and microglia activation can express both pro- and anti-inflammatory cytokines, therefore contributing to both neuroprotective and detrimental effects

(Hernandez-Ontiveros et al., 2013; Pekny et al., 2014). Overexpression of the neuroinflammatory response in the form of prolonged microglial and astroglial activation is an increasingly described feature of TBI. Mouse models have shown resting astroglial activation, that is, glial fibrillary acidic protein (GFAP) immunoreactivity at 6 months after a single mTBI. However, this evolved into mild reactive astrogliosis with thickened cell processes and hypertrophic cell bodies by 12 months after injury, a development not mirrored in their sham counterparts (Mouzon et al., 2014). Further, immunohistochemistry on human autopsy brain sections of mild to severe TBI patients have shown maximal microglial activity around 3 months with return to control levels only after several years (Smith et al., 2013). Interestingly, microglia presented distinct morphological phenotypes. Using PET data, elevated glia-mediated inflammation was detected in eight of the 12 brain regions examined in a young and healthy cohort of active and recently retired National Football League players with a history of repetitive concussion, compared with matched controls (Coughlin et al., 2017). Of note, the athletes reported a mean of 7 years since the last concussion and were free of any neuropsychological deficits. Another study observed high cerebrospinal fluid concentration of astroglial activation markers (GFAP, YKL-40) and altered amyloid metabolism in professional players with post-concussion syndrome for more than 1 year due to multiple concussions, compared with controls (Shahim et al., 2017).

Finally, the experimental data from various neurological disorders suggest that if neuroinflammation is not resolved within the post-acute phase, the benefits of reactive gliosis at that stage can trigger inhibitory effects on neuroplasticity and regeneration at chronic stages (Pekny et al., 2014).

Persistent neuroinflammation as a secondary process in the injured brain is an important cellular mechanism that may be linked to the exaggerated cortical thickening found in the PO subgroup. If confirmed by future studies, treatments in the form of pharmacological intervention or brain stimulation should try to inhibit or at least to attenuate the neurotoxic response of the resident immune cell of the central nervous system and its progression. In this context, cortical thickness can be a potential biomarker for the measurement of treatment-induced structural changes and therapeutic efficacy. The current results point the way toward strategies that specifically target the PFC, in particular of susceptible individuals at risk of chronic neurobehavioral symptoms.

Our study has some limitations. First and foremost, the sample size of the PO subgroup was restricted to six patients, reducing the statistical power to observe significant trajectory differences between the clinical subgroups or subgroup differences at Visit 2. Therefore, all

findings reported for the PO subgroup have to be interpreted with great caution and accordingly considered as preliminary results. Second, the study would have benefited from more scan points (at 3 and 6 months) to permit closer monitoring of cortical changes over time and to allow clearer understanding of the early development of GO and PO after mTBI. A third limitation of this study is the possible effect of head motion on thickness estimates of cortical regions that introduce image artifacts and reduce reliability (Reuter et al., 2015). Although no on-line motion correction procedure during MRI acquisitions has been applied, motion estimates of the same sample of 98 participants were available for rsfMRI and DTI data (Dall'Acqua et al., 2017). These measurements did not reveal any statistically significant differences between time-points within groups nor between groups at both time-points. Therefore, we assumed that there also were no significant head motion differences in the T1-weighted images.

In conclusion, this is the first investigation that has used surface-based morphometry to explore cortical evolution over 1 year after mTBI. The findings indicate that the PFC, a vulnerable region that relies on efficient long-distance fibers, plays a key role in long-term repair that may well be supported by practice or experience after the injury. Future research should be aimed at establishing the relationship between cortical thickness and the underlying histological processes to facilitate the development of effective interventions.

# 5 General discussion

To the best of my knowledge, the longitudinal project of this PhD thesis is the first work that investigated spontaneous neuroplasticity over the course of one year after mTBI using three distinct MRI techniques as well as different cognitive measures. The project involved two studies, which have been explicitly discussed in Chapters 4.1 and 4.2.

In this final chapter, the main results of Studies I and II are briefly summarized and discussed with respect to each neuroimaging modality and the relationship between them. The chapter then reviews important aspects of the dissociation between neural and cognitive recovery and of clinical outcome and its associated risk factors. Finally, the general discussion ends with some implications for the management and possible treatment strategies of the mTBI population together with challenges and suggestions for future work.

## 5.1 Summary of findings

A longitudinal design was applied to monitor the dynamic processes of brain reorganization and, more specifically, to elucidate alterations in functional and structural brain connectivity and in cortical thickness after a single mTBI. In addition to group-by-time interaction analyses, the same neuroimaging measures were tested cross-sectionally in both the acute phase (Visit 1, < 7 days post-injury) and the chronic phase of mTBI (Visit 2, 1-year post-injury). Furthermore, functional and structural brain alterations over time were explored in association with long-term cognitive changes. Finally, neural patterns corresponding to the outcome-specific trajectories of the subclinical groups (good versus poor recovery) were examined longitudinally.

In general, it is important to reiterate that the scant existing literature, previously discussed in the empirical studies, has reported very divergent findings about whether mTBI induces decreases or increases in structural connectivity, functional connectivity, and cortical thickness and about the location of the anomalies. In fact, the existing literature focused their analyses on a much shorter post-injury time and did not include interaction analysis, making it difficult to formulate detailed hypotheses.

### 5.1.1 Functional connectivity

Network analysis at the whole-brain level revealed reduced functional connectivity in the acute phase compared to healthy controls (see Study I). This functional hypoconnectivity was identified in a network broadly similar to the classical DMN and comprised central structures

such as the precuneus, PCC, ACC, TP, and STG.

The functional connectivity increased significantly over time within this specific network (selective interaction), but differences were still evident at the end of the year. Moreover, the partial recovery was clinically relevant and related to performance improvement in working memory and divided attention. After the patient group was dichotomized by symptom severity, the GO subgroup showed a very steep increase in functional connectivity, while the recovery trajectory of the PO subgroup was rather flat. However, no significant differences were observed at Visit 2 between the PO and GO subgroups.

The application of a longitudinal whole-brain interaction analysis to detect additional changes emerging after Visit 1 yielded evidence of further connections with decreased functional connectivity. In contrast to the finding of the selective interaction, these supplemental connections completely normalized, and this happened equally to patients in the PO and the GO subgroups.

### 5.1.2 Structural connectivity

Network analysis at the whole-brain level revealed increased structural connectivity in the acute stage compared to healthy controls (see Study I). The structural hyperconnectivity was identified in an extended frontotemporoparietal network including precuneus, PCC, ACC, and TP, commonly assumed to represent central nexus of the connectome.

The structural hyperconnectivity decreased significantly over time within this network (selective interaction), but the differences were still evident at the end of the year. Moreover, the recovery was also clinically relevant and related to performance improvement in verbal memory and divided attention. At Visit 2, no significant difference was found between the PO and GO subgroups.

The application of the longitudinal whole-brain interaction analysis, similarly to the functional analyses above, also yielded evidence of further connections with increased structural connectivity. These additional connections entirely restored in the chronic phase with a tremendous decrease in connectivity.

### 5.1.3 Cortical thickness

Analyses of T1-weighted structural data yielded no differences between mTBI patients and healthy controls at Visit 1 (see Study II). However, the longitudinal interaction analysis at the whole-brain level showed a considerable increase in cortical thickness in the patients. This thickening comprised almost symmetric clusters covering the bilateral APFC, MPFC, ACC,

and OFC. One year later, the morphology of the PFC remained still altered, although the area of cortical thickening was less extensive.

Patients in the PO subgroup exhibited a stronger cortical thickening over time than patients in the GO subgroup, whose modest thickening rather suggest a lack of the maturational thinning usually observed in the healthy population (Wierenga et al., 2014; Amlien et al., 2016; Walhovd et al., 2016). At Visit 2, probably due to the small size of the PO subgroup, there was no significant difference between the clinical subgroups.

The modest thickness increase seen in the asymptomatic patients, particularly together with cognitive recovery in memory and executive functions, may indicate restorative neuroplasticity. Conversely, the robust thickening detected in the symptomatic patients, especially when associated with worsening in divided attention, may signal a protracted state of neuroinflammation and therefore suggest maladaptive plasticity.

### 5.1.4 Analogies and differences in the multimodal findings

The comparison of both studies revealed some analogies, but also some differences. Overlapping nodes of both functional and structural altered connectivity values at Visit 1 involved the following 12 structures: bilateral precuneus, bilateral PCC, bilateral ACC, right STG, right SMA, right parahippocampal gyrus, right amygdala, left Heschl's gyrus, and left TP (Figure 4.1.6, Study I). Moreover, these regions were inversely related to each other: the stronger the functional hypoconnectivity was, the stronger was the structural hyperconnectivity. All 12 functional-structural nodes except for three were engaged in the evolution of recovery, but in contrast to the acute phase, the normalization of functional connectivity was no longer related to that of structural connectivity.

Changes in cortical thickness were not observed at Visit 1 but exclusively in the subsequent weeks and months (Study II). Additional exploratory analyses between the findings of Study I and Study II identified a significant positive correlation between the neuroreparative processes in the structural subnetwork 2 concentrated in the right hemisphere and in the right frontal cluster (unpublished data).

The anomalous increase in cortical thickness found in the PFC clusters (see Study II) was also detected in the ACC, suggesting a key role of this structure in the mTBI population. The fact that the ACC is vulnerable to brain damage independent of the impact location and the chosen MRI sequences support the understanding of the ACC as a densely anatomically connected region with a high central position in the overall network, crucial for communication and global integration (van den Heuvel and Sporns, 2013). This highly interconnected hub

node is a member of the so-called “rich-club” organization and is considered a highest-ranking node (van den Heuvel and Sporns, 2011; Stam, 2014). Moreover, the ACC is classified as a connector hub, since it is strongly linked to nodes in other modules, has a high number of long-distance connections, and participates in multiple functional networks such as the DMN and the salience network (Hagmann et al., 2008; van den Heuvel and Sporns, 2013). The ACC is especially active in situations demanding cognitive control and is involved in monitoring, motivation, effort, reward, and chronic pain conditions (Leung et al., 2016b; Shenhav et al., 2016).

Overall, the process of neural restoration was not yet complete after 1 year in any of the neuroimaging modalities. Therefore, it seems plausible that neuroplasticity after mTBI necessitates more than 1 year to return to the level observed in healthy controls. In general, the neural alterations were more pronounced in patients who recovered poorly. This fits with established literature, which shows that outcome-specific trajectories differ depending on symptom severity (Messe et al., 2011; Messe et al., 2012). One further commonality between Study I and II is that the recovery observed with MRI was clinically relevant and paralleled by improvements in cognitive performance.

### 5.1.5 Advantage of utilizing multimodal neuroimaging techniques

As mentioned in the theoretical background, this thesis focused on the integration of a variety of neuroimaging methods within the same framework. Study I combined information about functional and structural connectivity using rsfMRI and DTI. Study II added data gained from structural T1-weighted images combined with surface-based morphometric analyses and led to analogous conclusions, highlighting the importance of the results obtained from Study I (see section 5.1.4).

Multimodal MRI techniques facilitate neuroimaging research by overcoming the limitations of any single modality and by investigating the associations of findings from diverse sources of information (Liu et al., 2015a; b). Multiple neuroimaging biomarkers have important implications in mTBI research, as they allow better capturing and understanding of complex, multifaceted lesion-induced neuroplastic potential, especially restorative and maladaptive neuroplasticity. Nowadays, it is hard to imagine medical and scientific progress in mTBI without combining multiple modalities.

## 5.2 Dissociation between neural recovery and cognitive recovery

What both empirical studies presented in this thesis have in common is the dissociation of cognitive from neural (functional and structural) normalization. On one hand, the recovery from initial cognitive impairments was complete, at least when comparing scores of the cognitive tests 1 year after injury with those of healthy participants. On the other hand, the recovery of functional and structural connectivity of the disrupted networks at Visit 1 was only partial, and the reorganization of cortical thickness was still ongoing 1 year after mTBI.

These observations suggest that the resolution of cognitive deficits seems not to coincide with neural baseline. Similar to this thesis, previous mTBI studies have documented neuroimaging dysfunctions, especially anomalies in resting-state connectivity and task-related activations, in spite of intact neuropsychological performance (McAllister et al., 2006; Mayer et al., 2011; Chen et al., 2012). For instance, in mTBI patients relative to their controls, one study reported functional hypoconnectivity within the DMN and hyperconnectivity between the DMN and prefrontal cortical areas that persisted across a 4-months period (Mayer et al., 2011). However, deficits on cognitive tests were absent.

A body of literature provides evidence that the concussed brain possesses the ability for *cognitive compensation.* The mechanism of recovering the function may involve (i) new organization of existing brain network and/or (ii) recruitment of new areas within the same network associated with the given function and/or (iii) recruitment of new areas inside an alternative network (Sanchez-Carrion et al., 2008; Chen et al., 2012).

In this thesis, the process of neural reorganization after mTBI seems to require more than 1 year. Nevertheless, it is unknown whether compensatory changes may lead to *transient* functional and structural modifications with ultimate full return to normal state or whether recovery-related changes should be rethought and viewed rather as *permanent* neural modifications.

Another issue that deserves consideration is related to the appropriateness of classical neuropsychological assessments as measures of possible mTBI-induced sequelae in the chronic stage. Conventional neuropsychological instruments are certainly sensitive enough to detect cognitive deficits after moderate to severe TBI or in the acute phase after mTBI (Collie and Maruff, 2003; Rabinowitz et al., 2013). However, standard cognitive assessments may underestimate the subtle residual dysfunctions associated with chronic mTBI or at least underestimate the time needed to completely recover. Previous research has recognized that the ability of neuropsychological assessments to disclose mTBI-related cognitive anomalies

precisely decreases as time elapses (Iverson, 2005). Furthermore, no cognitive test battery is universally accepted as an objective assessment of post-concussion symptoms.

In this thesis, the comparison between the entire patient group and their healthy controls on cognitive test scores at Visit 2 revealed no significant differences after the adjustment for multiple comparisons (FDR correction). The same finding was obtained when the group comparison was conducted between the PO subgroup (n = 6) and the controls.

The possibility that normalization in brain function and structure may be more limited or lag behind the normalization of cognitive functioning highlights the need for objective markers for recovery status. Some investigators have proposed *impaired oculomotor and visuomotor functions* as markers associated with long-term post-concussion sequelae (Heitger et al., 2008; Heitger et al., 2009; Subotic et al., 2017). Ocular motility in general and saccades in particular rely on a complex network involving cortical and subcortical structures, including frontal areas, superior colliculus, basal ganglia, and the cerebellum (Ramat et al., 2007). Measurements of eye movement function may serve as an early predictor of the development of PCD (Heitger et al., 2008; Heitger et al., 2009; Cifu et al., 2014).

In a pivotal study, subjects with symptomatic mTBI at ~140 days post-injury but otherwise unremarkable neuropsychological profiles performed significantly worse on anti-saccades, self-paced saccades, memory-guided sequences of saccades, and oculomotor smooth pursuit than patients with good recovery (Heitger et al., 2009). These oculomotor deficits are critical since they can result in the inability to inhibit erroneous responses (response inhibition) and in difficulty in disengaging attention. In addition, the poor recovery group also showed impaired performance on a variety of eye movement paradigms under subconscious control (Heitger et al., 2009). The evidence of anomalous subconscious oculomotor function, indicative of problems in subcortical processing, suggests that post-concussion symptoms do not have a merely psychological substrate, but are also of biological nature. The same authors found that poorer eye movement function was better correlated with limitations in activities of daily living than poorer neuropsychological function (Heitger et al., 2009). Another recent investigation found enhanced sensitivity of saccadic measures towards neurocognitive measures in patients with a remote history of mTBI, suggesting that saccadic impairment may mirror chronic consequences of mTBI that traditional tools are unable to detect (Hershaw et al., 2017). It is worth mentioning that the evaluation of eye-movement outcome has the advantage of being independent from intellectual ability and psychological factors such as depression, in contrast to classical cognitive tests of neuropsychological assessment batteries.

As an alternative to high-technology eye-tracking, other work has adopted more low-

technology evaluation approaches. For example, there is growing interest in the King-Devick test as a rapid and simply administered yet sensitive assessment tool for mTBI (Oride et al., 1986; Galetta et al., 2011b; Subotic et al., 2017). This test has a high test-retest reliability. It requires participants to read numbers quickly from left to right on a series of three test cards, which become gradually more difficult to read (Galetta et al., 2011a; Rizzo et al., 2016). This number-naming tool identifies impairment of saccades, eye movements, and visual fixation.

Frontal regions have been proposed to be of particular importance for the accuracy and velocity of saccades. The suboptimal functionality of DLPFC, ACC, frontal eye field, and cingulate eye field may thus be an underlying cause of saccadic anomalies (Pierrot-Deseilligny et al., 2002; Pierrot-Deseilligny et al., 2005; Heitger et al., 2009; Choi et al., 2014). This hypothesis is interesting, since exactly the same brain regions have been speculated in this thesis to be the least likely to fully recover after mTBI.

## 5.3 Clinical outcome and risk factors associated with poor recovery

The mTBI sample investigated in this thesis was dichotomized into complete recovery (good outcome; GO) and incomplete recovery (poor outcome; PO) at both 1 week and 1-year post-injury. At 1 week, approximately 30% of the sample met the PCD criteria. Of those, 60% (n = 9) improved and 40% (n = 6) remained symptomatic at 1 year. Despite the significant decrease in the number of symptoms throughout the year, six patients continued to report pronounced cognitive, physical, and emotional symptoms. Headache, sleep disturbance, fatigue, and forgetfulness were the sequelae most frequently reported by these six patients.

Studies I and II were not capable of identifying significant differences between the neural recovery trajectories of the good and poor outcome subgroups. The enrolment of more patients to collect a larger sample falling in the PO category may allow us to determine whether some results that were only marginally significant can show a stronger effect.

Given the complex processes behind the etiology and persistence of PCD, it is unlikely that a simple explanation or a single prognostic factor can account for the heterogeneity in outcome. Each brain injury is unique and each subject reacts differently to injury, even if the injury mechanism is the same. In addition, the severity of mTBI characterized by indicators such as duration of PTA and of LOC had no long-term prognostic value (Carroll et al., 2004b; Silverberg et al., 2015; Waljas et al., 2015). A number of risk factors other than the traumatic brain injury itself may better explain the variability in recovery rates.

The next paragraph discusses several pre-, peri-, and postinjury risk factors that have been suggested to contribute to the various recovery-related trajectories. These risk factors

encompass demographic, psychological, personality, psychiatric, genetic, psychosocial, financial, and physical variables.

### 5.3.1 Demographic factors and cognitive reserve

Demographic variables such as gender, age, and years of education appear to be important determinants of long-term outcome. Many reviews and studies have identified an association between female *gender* and worse prognosis (King, 2014; Silverberg et al., 2015). However, it remains unclear whether the relation between gender differences and increased risk of PCD is real or whether it is distorted by more frequent and more honest reporting of females. In fact, other studies have not found any difference in the incidence of prolonged recovery between males and females (Yuh et al., 2013; Ganti et al., 2014). In the PCD subgroup of this thesis, gender distribution was the same (3 male and 3 female).

Further, *age* may also give rise to individual outcome. Evidence indicates that adolescents and older adults appear to be more vulnerable for long-lasting sequelae (Ponsford et al., 2000; Lovell et al., 2003). However, results for this variable are also heterogeneous and other studies have reported a generally more favorable recovery in patients aged over 65 years (Mosenthal et al., 2004; van der Naalt et al., 2017). In this thesis, the factor of age was similar between the clinical subgroups, that is 34 years for the GO and 41 for the PO patients.

In contrast, the *education level* differed trend-wise between the GO subgroup (12.8 years of formal education) and the PO subgroup (10.8 years of formal education). Indeed, the demographic factor that seems most able to account for the widespread variability in outcome after mTBI is education. Lower education or intellectual level is associated with worse early functional status and is a risk variable for protracted symptoms following mTBI (Teasdale and Frosig, 2013).

The same relation is true for *intelligence*, another measure related to education. Lower pre-injury IQ predicts increased likelihood of not returning to full-time work in the aftermath of the injury (Vanderploeg et al., 2003). This is consistent with the data of this thesis, which yielded a significantly lower IQ score in the PO subgroup (IQ of 89.3) than in the GO subgroup (IQ of 102.8).

The concept of *cognitive reserve* has been proposed to explain why individuals with elevated IQ and education are more capable of sustaining greater injuries without prolonged clinical impairments (Stern et al., 1992; Alexander et al., 1997). Cognitive reserve designates the brain's ability to use its resources efficiently to cope with neurological damage, whether in terms of functional capacity or brain morphology (Marques et al., 2016). The cognitive reserve

model states that subjects with higher cognitive reserve capacity can deploy their neural resources in a more flexible or efficient way to overcome the challenges of brain damage (Stern, 2009). A recent study investigated functional connectivity networks associated with cognitive reserve (Marques et al., 2016). It found that higher education level was associated with greater functional connectivity in a large cortical network and that people with greater cognitive reserves showed increased network efficacy that might be reflected in an increased capacity for parallel information processing (Marques et al., 2016).

In this thesis, the wide variation in educational and intellectual level among the patient sample may contribute to individual differences in recovery trajectories.

### 5.3.2 Psychological and personality factors

Other indicators that may help in the identification of poor recovery after mTBI are of *psychological nature*, such as maladaptive beliefs, perceptions of the injury, attribution bias, and psychological distress. In general, studies suggest that patients with expectations of lasting severe consequences, stronger injury identity beliefs, and higher levels of emotional distress in the acute phase have greater odds of an unfavorable outcome (Ozen and Fernandes, 2011; Snell et al., 2013).

Susceptibility to developing persistent symptoms might also be influenced by *personality* factors. A personality able to deal with new situations, stressors, and emotions adequately will be more protected from long-term sequelae. It should be emphasized that interindividual differences in adaptation are reflected by the use of different *coping styles* (van der Horn et al., 2015a). Measures of coping style have received substantial attention in recent research as they have a high predictive power for recovery. Active coping, which includes problem-focused and emotion-focused coping strategies, is mainly associated with a beneficial outcome (van der Naalt et al., 2017). Conversely, passive coping, which entails avoidance and denial of problems and a bias toward negative emotions, has been related to negative outcome. Furthermore, coping styles are partially dependent on *feelings of self-efficacy*. Subjects with high belief in their self-efficacy have greater and more optimistic trust in their abilities to deal with difficult circumstances than subjects with low belief in their self-efficacy (Taylor and Stanton, 2007; Scheenen et al., 2017). A new study found that high levels of self-efficacy were related to a more active coping style and low levels of self-efficacy to a more inadequate/passive coping style in the mTBI population (Scheenen et al., 2017). The same authors also found that six coping styles (active coping, distraction, avoidance, seeking social support, expression of emotions, positive reframing) were variable throughout the first year after injury in contrast to

the passive one, which demonstrated no change over time (Scheenen et al., 2017). Early passive coping therefore has the potential to predict chronic posttraumatic complaints.

For instance, an avoidant coping style that is hypothesized to be partly responsible of headaches after mTBI is *cogniphobia*. Cogniphobia refers to an excessive avoidance of mental exertion because of fear of eliciting or worsening a headache (Schmidt, 2003). Besides mentally demanding tasks, patients with severe cogniphobia also tend to avoid physical activities. This behavior can lead to significant lifestyle changes, functional disability, and other psychological comorbidities. Recent findings showed that mTBI patients with greater headache severity indicated greater avoidance of mental exertion (Silverberg et al., 2017). Interestingly, this association was found to be particularly strong for participants with low educational level. Finally, it is important to keep in mind that maladaptive coping is associated with increased incidence of psychiatric factors (see 5.3.3).

### 5.3.3 Psychiatric factors

As mentioned in the Introduction, psychiatric variables such as pre- and/or post-injury psychiatric disorders have also been identified as risk factors for unfavorable outcome after mTBI. It is widely accepted that pre-existing and early mental health problems such as depression, anxiety, and post-traumatic stress disorder are robust prognostic factors of persistent PCD (Ponsford et al., 2012; McCauley et al., 2013). In this research, we rigorously excluded patients with obvious premorbid affective problems based on their clinical assessment during recruitment. One patient was subsequently excluded after Visit 1 because of a psychiatric history. Nevertheless, we cannot completely rule out the possibility that undiagnosed psychiatric disease or pre-existing psychological conditions may account for the variable response to brain injury.

### 5.3.4 Genetic factors

Genetic influence is likely to play a part in the development of persistent sequelae and has received increased attention in recent years. Emerging literature on genetic factors in TBI propose three broad frameworks in which genotype could modulate outcome: (1) repair processes, (2) pre-injury traits and cognitive reserve, (3) interaction between trauma and genetic susceptibility (McCrea et al., 2017).

Two genetic studies by Merritt and coauthors found a relationship between the e4 allele of the apolipoprotein E (APOEe4) gene and reporting of post-concussion symptoms, such as more severe headache after sustaining a sports-related concussion (Merritt and Arnett, 2016;

Merritt et al., 2016). One study showed that APOEe4-positive athletes expressed significantly greater physical and cognitive symptomatology than APOEe4-negative athletes at 3 months post-injury (Merritt and Arnett, 2016). The possession of APOEe4, a protein that has also been implicated in moderate to severe TBI and in Alzheimer's disease, may confer greater risk for experiencing poor outcome following mTBI. However, the level of evidence for genetic assessment as a clinical tool in the management of mTBI is currently low and warrants future research.

### 5.3.5 Other factors

Numerous other factors, including psychosocial, financial or physical factors, may render individuals more or less resilient to the effects of mTBI.

Poor *social support* or possible *financial gain* through litigation or compensation has been found to contribute to the persistence of posttraumatic symptoms. A particular strength of the current research is that motivational variations were assessed as part of the neuropsychological evaluation and yielded good compliance for all participants. Therefore, outcome distortion due to lack of effort or deliberately poor test performance could be excluded.

Further, a *physical* condition that has been identified as negatively influencing the development of long-term sequelae is the absence of alcohol consumption at the time of injury (van der Naalt et al., 2017). Alcohol intoxication in the emergency department might be protective against adverse outcome since it attenuates the memories of the traumatic event. Other physical factors that have been found to modulate mTBI outcome are prior history of migraine, extracranial injury, and musculoskeletal attributes. For example, there is limited but positive evidence that the co-occurrence of extracranial bodily injuries are significant predictors of PCD (Waljas et al., 2015). Further, well-developed neck and shoulder musculature may protect against high rotational acceleration of the head. Consequently, men’s thicker neck muscles have been proposed as a variable that may explain why men appear to be less vulnerable to adverse recovery than women (Rabinowitz et al., 2013).

In conclusion, several neurobiopsychosocial factors appear related to the individual outcome after mTBI, and the discovery of these factors is an important area of research worldwide. At present, no prognostic model can accurately identify the various recovery trajectories. The development of long-term sequelae is likely multifactorial, influenced by the cumulative and/or interactive effects of diverse risk factors. The elucidation of these factors will enable the prevention of unfavorable outcomes and reduce their unacceptable incidence.

## 5.4 Implications for the management of mTBI patients and therapeutic strategies

The findings of this thesis have important clinical implications. These might be used to update the management of mTBI and to suggest therapeutic interventions that promote long-term neurorestorative effects. Both studies found that a year is not enough time for the brain to return to baseline and that endogenous mechanisms alone may be not sufficient to completely restore the central nervous system after mTBI. It is therefore necessary for clinicians to adjust their expectations about the natural speed of their patients' neural recovery. Next, it is essential to emphasize the importance of preventing further mTBI during the 12 months post-injury. A detailed evaluation of post-concussion symptoms by means of the RPQ is also recommended in the very acute phase – ideally in the emergency room, as this questionnaire is not something that is routinely completed at present. The RPQ is a useful instrument for identifying those at risk for incomplete recovery, and the medical stuff should take note of which trauma-induced symptoms are elevated and may need to become a focus of intervention. Offering proactive assistance, appropriate advice, and effective treatment may help to ameliorate the outcome of this difficult-to-treat population. To date, classical treatments available for prolonged complaints vary greatly depending on the primary symptoms reported, but there is no consensus on the best treatment strategy. Physicians treat most of this minority with analgesics, narcotics, triptans, anti-anxiety medications, or antidepressants, and refer about 40% to psychological intervention (Mittenberg et al., 2001). Unfortunately, current pharmaceutical treatments have not only been shown to be ineffective but often entail adverse psychosomatic and long-term abusive side effects (DiTommaso et al., 2014; Lucas et al., 2014; Carpenter et al., 2015). Therefore, alternative non-pharmaceutical options should be assessed and validated.

Noninvasive brain stimulation techniques, such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) may offer promising therapy options for alleviating chronic mTBI-related symptoms (Dhaliwal et al., 2015; Li et al., 2015). Due to their ability to modulate neuron firing and so inhibit maladaptive plasticity, these novel interventions are worth exploring (Villamar et al., 2012). Both neuromodulatory tools produce effects at network level that are capable of increasing synaptic strength and modulating neurotransmission (Funke and Benali, 2011; Li et al., 2015). Furthermore, they have been credited with enhancing recovery in various neurological (such as severe TBI) and psychiatric disorders.

### 5.4.1 Transcranial Magnetic Stimulation (TMS)

The first TMS devices were approved by the US Food and Drug Administration (FDA) in 2008 and were subsequently approved for treating migraines and treatment-resistant major depressive disorders after receiving support from randomized studies and meta-analyses (Leung et al., 2009; Horvath et al., 2011; Lefaucheur et al., 2014). TMS induces electric activity in neural tissue via the use of rapidly changing magnetic fields that pass through the skull. Electrical pulses stimulate populations of neurons, axons, and dendritic circuits, and the repeated flow of current generates long-term modifications in neural activity that can reorganize the network after injury (Li et al., 2015). Low-frequency rTMS (0.2-1 Hz) is recognized to reduce cortical excitability, while high-frequency rTMS (≥ 5 Hz) tends to increase neural excitability in the stimulated area.

So far, Koski et al. (2015) conducted the largest intervention study; this aimed to test the benefit of high-frequency rTMS (10 Hz) on patients suffering from post-concussion sequelae at least 6 months after mTBI (Koski et al., 2015). Fifteen patients received 20 sessions (over 4 weeks) of stimulation over the left DLPFC, a region whose treatment has proven to relieve depressive symptoms in major depression (Lam et al., 2008; George et al., 2010; O'Reardon et al., 2010). Overall, PCS scores declined significantly in 75% of the patients; at symptom level, a decrease in the ratings of headaches showed the most reliable improvement. Clear support for the efficacy of rTMS was also derived from the fMRI scans conducted after the intervention; these yielded increased activation in the DLPFC and increased deactivation of the ACC during a working memory task than prior to treatment (Koski et al., 2015). The results of a 3-month follow-up demonstrated that these improvements may be of limited duration, indicating that further maintenance sessions are required. The absence of a control group was the main limitation of this pilot study. The authors concluded that rTMS is safe, well tolerated and can be effective in treating chronic symptoms of mTBI.

Two subsequent studies showed the effectiveness of rTMS in alleviating chronic mTBI-related headache (Leung et al., 2016a; Leung et al., 2017). The first study targeted the left motor cortex (3 stimulation sessions within 1 week), while the second study targeted the left DLPFC (4 stimulation sessions within 1 week). Both randomized studies delivered high-frequency stimulation (10 Hz) and demonstrated a significant reduction in the intensity and overall frequency of persistent daily headache. In contrast to the control group, this effect appeared to be present in the treatment group not only at the 1-week post-treatment evaluation but also up to 4 weeks post-treatment (the maximum duration of the study). These two rTMS protocols appear to be feasible in clinical practice as a sustainable therapy in managing

persistent headache after mTBI, since a short period of treatment may improve patient compliance and is not time consuming for most clinicians.

Besides these three clinical studies, the literature offers only single case reports with severe TBI without control groups to verify the findings (Bonni et al., 2013; Kreuzer et al., 2013; Nielson et al., 2015). While mTBI may not pose a greater seizure risk than the general population, severe TBI is generally regarded as a contraindication for rTMS studies due to potential seizure risk (Annegers et al., 1980; Annegers and Coan, 2000).

### 5.4.2 Transcranial Direct Current Stimulation (tDCS)

tDCS applies a constant low electrical current (1-2 mA) for 10-20 minutes between two large scalp electrodes (ca. 20 cm2). The current streams from the electrode of interest, or anode, to the control electrode, or cathode (Nitsche et al., 2008). When the electrode installed over the brain area of interest is an anode, the cortical excitability of the targeted tissue is increased, whereas when the electrode is a cathode the cortical region is inhibited. It is generally accepted that a single tDCS session of about 10 minutes can lead to post-stimulation excitability lasting longer than 1 hour, with multiple sessions resulting in long-term effects (Nitsche and Paulus, 2001; Boggio et al., 2007).

As of 2018, the therapeutic use of tDCS has not been approved by the US FDA, but tDCS is approved by the European Conformity for the treatment of major depressive disorders in Europe with level B evidence (probable efficacy) (Lefaucheur et al., 2017). There is increased interest in the application of tDCS due to its permanent technical progression, low cost, and facility and safety of use. According to a recent review, tDCS does not pose the same risk of seizure in the treatment of severe TBI patients as rTMS (Bikson et al., 2016). Therefore, more randomized controlled studies have currently examined tDCS than TMS in severe TBI.

Preliminary examinations demonstrated the potential of this technique for improving chronic cognitive impairments after severe TBI, focusing primarily on the restoration of attention and memory (Kang et al., 2012; Ulam et al., 2015; Sacco et al., 2016; O'Neil-Pirozzi et al., 2017). For example, a recent randomized controlled trial with chronic TBI patients showed that a 5-day period of treatment with anodal stimulation over the DLPFC (2 mA for 20 min) followed by computer-assisted cognitive training resulted in a significant improvement in divided attention performance (Sacco et al., 2016). The site of the treatment (left or right DLPFC) varied depending on each patient’s area of damage. The effect was long lasting; the attentional enhancement was maintained in the 1-month follow-up evaluation. In addition, fMRI data detected processes of intervention-related neuroplasticity in a post-treatment

decrease in cerebral activation during the divided attention condition (Sacco et al., 2016). Other crossover studies applied the same stimulation parameters as above but with just a single session of anodal stimulation over the left DLPFC. These produced immediate benefits in reaction times and working memory performance (Kang et al., 2012; O'Neil-Pirozzi et al., 2017).

No specific studies looking at patients with mTBI were found in the literature, so the therapeutic effect of tDCS on persistent symptoms remains to be clarified. However, a protocol for a randomized crossover trial for mTBI with a clinically defined PCD has recently been published (de Amorim et al., 2017).

In conclusion, additional investigations are required with larger sample sizes and controlled, randomized designs matched for age, education, gender, and time since injury to ascertain the true efficacy of rTMS and tDCS on chronic symptoms following mTBI. Although the cellular substrates underlying the mechanisms of these neuromodulatory techniques remain poorly understood, these tools have shown great potential for suppressing maladaptive plasticity.

The question of which cortical target is the most effective to be stimulated has yet to be resolved. Among several locations of treatment, almost all the clinical trials described above focused on the DLPFC. This region is not only pivotal in the performance of attention and working memory tasks but also has a large number of long-range connections and is in turn crucial for global neural communication (Bullmore and Sporns, 2012). Study II also found a strong anomaly in the DLPFC in form of excessive cortical thickening in the PO group. This might be avoided with the use of a tDCS treatment. Alternatively, the choice of treatment location may be tailored to the specific concerns of each individual case. Given the heterogeneity of the damage and the diverse sets of symptoms in the mTBI population with PO, an undifferentiated protocol for all of them is likely not ideal.

Because PCD is highly complex and influenced by a range of factors, a combination of different neurorehabilitation approaches may maximize the response to brain stimulation. For example, coupling non-invasive stimulation with traditional cognitive training or psychotherapy could increase the benefit of each single treatment. Whether brain stimulation tools are suitable for increasing functional connectivity or decreasing structural connectivity or cortical thickness has yet to be investigated. If they are, these measurements could be selected as sensitive biomarkers to identify training-related neuroplasticity in mTBI.

### 5.4.3 The role of glia

The mechanism of action of rTMS and tDCS on glial cells and on the interaction between neurons and glia is largely unknown. In the human brain, glia represents about 50% of the cells, and although these cells cannot produce action potential as neurons do, they are electrically active, which allows sensitivity to external electrical stimulation (Gellner et al., 2016). It is increasingly recognized that glia structures participate in neuroplasticity, and their important role can no longer be ignored.

(i) Imaging and histological assessments reveal that tDCS may induce neuroplasticity through the involvement of glia. TDCS may induce electrical effects on astrocytes with consequent changes in neurotransmission (Gellner et al., 2016). In addition, tDCS may also modulate the responses of microglia (Braun et al., 2016). For instance, high-intensity anodal and cathodal multi-session tDCS of the rat cerebral cortex has been shown to activate microglia (Rueger et al., 2012).

(ii) Promising evidence for the effective influence of glia in response to rTMS has also been shown in an animal model, where rTMS seems to attenuate inflammation and facilitate neurological recovery after brain damage (Sasso et al., 2016). The study yielded reduced glial activation and demonstrates the potential of rTMS as an anti-inflammatory treatment. As discussed in Study II of this thesis, prolonged reactive proliferation of astrocytes and microglia in the chronic phase is a candidate mechanism explaining the exaggerated cortical thickening observed in the PO subgroup.

Future studies are necessary to examine the specific effects of rTMS and tDCS on glia in neurological conditions and to boost therapeutic efficacy.

## 5.5 Challenges and future directions

This thesis focused on spontaneous neural reorganization throughout the first year after a single mTBI. This growing clinical population is considered challenging to investigate at both methodological and conceptual levels. On the one hand, the damage inflicted frequently involves more than one functional and structural brain region. On the other hand, the damage differs among patients depending on impact characteristics such as mechanism of injury, location, direction, and force. A longitudinal design combined with the application of multiple neuroimaging modalities offer a unique opportunity to gain further insight into the tremendous neuroplasticity of mTBI.

In the near future, neuroimaging techniques will continue to improve, with faster scanning speeds, higher spatial and temporal resolutions and advanced hybrid scanners able to

simultaneously record multimodal data (e.g. MRI/EEG, MRI/PET). Until now, the availability of computing methods that incorporate a unified pipeline for the analysis of heterogeneous data is very limited. The increasing interdisciplinary collaboration and synergies between computer scientists, engineers, psychologists, and physicians, it makes it likely that these constraints will be satisfied in the next years. A further challenge will be to translate improved neuroimaging technologies from research labs to clinical care contexts. Therefore, the development of user-friendly interfaces for facilitating multimodal longitudinal assessment that can fit into the clinical schedule should become a future trend.

Arriving at a consensual definition of PCD poses another challenge in the mTBI research community, and this difficulty complicates the comparison between findings from different studies. In the mTBI literature, patients scoring specific criteria are still assumed to be part of a monolithic group without internal differences. The development of accurate diagnostic criteria has to be a major aim for this research field. Even though much work has been done to understand the neural processes behind PCD, we are not yet able to find the cause and predict which patients continue to experience chronic sequelae.

The longitudinal dataset of this thesis limits robust analyses of the clinical patient subgroups. Since it is impracticable for any single center to enroll a large number of patients, multi-center studies or multi-institution consortia are crucial. Data sharing in neuroimaging is becoming increasingly common, and open science initiatives are just beginning to make MRI data freely available. The mTBI research community is encouraged by collaborative research networks such as the Concussion Assessment, Research and Education (CARE) Consortium under the leadership of Prof. McAllister and Prof. McCrea (www.careconsortium.net). They aim to comprehensively elucidate the natural history of concussion by integrating advanced neuroimaging, biological, clinical, biomechanical, and genetic markers.

This thesis represents a positive departure in mTBI by making longitudinal observations over extended follow-up period. For further research, I recommend more than two measurement points and a longer overall monitoring time. Intermediate and additional scans should ideally be performed at 1 week, 1/3/6 months and 2/3 years post-injury. Such a pattern of observations would ease identification of the period of increased risk for PO and its ultimate trajectory.

To remove bias in post-concussion symptoms reporting due to factors not specific to mTBI, future studies should include an orthopedically or other non-head injured control group instead of a healthy one.

Finally, it is important to recall that the vast majority of neuroimaging research in mTBI has thus far employed group analysis and that insufficient progress has been made towards individualized assessments. However, individualized analysis approaches are indispensable in this clinical population, first because each patient depicts a unique pattern of injury and second to guide individually tailored interventions. Mapping mTBI-induced damage into a patient-tailored framework or comparing the brain damage of a single subject with a normative atlas comprising anatomical data of healthy controls may both open interesting clinical scenarios (Irimia et al., 2012; Shenton et al., 2012; Irimia et al., 2014).

## 5.6 Concluding remarks

In conclusion, this PhD thesis uses a multimodal neuroimaging approach to demonstrate dramatic changes in brain connectivity and morphology following a single mTBI. Although the anomalies in network connectivity and cortical thickness were most pronounced in the acute phase, they were only partially reversible over 1 year. Highly connected hubs such as the PFC and the ACC emerged as the slowest structures to recover, suggesting analogies with the pathophysiology of more severe TBI. Despite cognitive recovery, the compensatory process of the injured brain required more time to return to baseline, if it did so at all, than previously assumed. The findings of this longitudinal work highlight the importance of monitoring the consequences of mTBI sufficiently frequently and over a sufficiently long period and emphasize the importance of preventing secondary brain injury.

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# Appendix

# A Supplementary material of STUDY I

## A.1 Supplementary Methods

### Neuropsychological assessment

The neuropsychological assessment focusing on the cognitive domains of attention, executive functions and memory included the following tests: (i) subtests Alertness (intrinsic and phasic), Go/Nogo, and Divided attention of the Test for Attentional Performance (TAP 2.2) (Zimmermann and Fimm, 2002). These tests measure attention, inhibitory control, and cognitive flexibility with varying complexity; (ii) German version (Von Aster et al., 2006) of the Backward Digit Span of the Wechsler Adult Intelligence Scale WAIS-III (Wechsler, 1997) to assess verbal working memory; (iii) Swiss adaptation (Balzer et al., 2011) of the Rey Auditory Verbal Learning Tests RAVLT (Strauss et al., 2006) to assess verbal learning (using the total number of words recalled across five trials) and short- and long-delay verbal recall. In addition, an estimation of the nonverbal intelligence level has been obtained by using the Wiener Matrizen-Test 2 WMT-2 (Formann et al., 2011), an adapted version based on Raven's progressive matrix test (Raven, 1958). The neuropsychological assessment also included a test measuring effort and symptom validity (Green's Medical Symptom Validity Test, MSVT). The MSVT (Green, 2004) is a brief automated verbal memory screening with several subtests designed to measure verbal memory and response consistency. In addition, two scales were used to assess emotional symptomatology: the German version (Hautzinger et al., 2006) of the Beck Depression Inventory 2$^{nd}$ edition BDI-II (Beck et al., 1996) was selected to control for manifestations of depression; and the German version (Margraf and Ehlers, 2007) of the Beck Anxiety Inventory BAI (Beck and Steer, 1993) was chosen to evaluate anxiety symptoms in response to mTBI.

### Magnetic resonance imaging data acquisition

MRI scans were acquired on a 3.0 Tesla Philips Ingenia whole body scanner (Philips Medical Systems, Best, The Netherlands) equipped with a transmit-receive body coil and a commercial 15-elements transmit-receive head coil array that is capable of sensitivity encoding (SENSE).

Resting-state functional MRI images were obtained using an echo-planar imaging sequence with an acquired spatial resolution of 3.0 x 3.0 x 3.0 $mm^3$ (acquisition matrix: 72 x 74 pixel, 45 axial slices) and reconstructed to a spatial resolution of 1.72 x 1.72 x 3 $mm^3$ (reconstruction matrix: 128 x 128 pixel, 45 axial slices). Further imaging parameters were: Field of view (FOV) = 220 x 220 $mm^2$; slice thickness = 3 mm; number of slices = 45; brain volumes = 140; repetition time (TR) = 2,220 ms; echo time (TE) = 15.19 ms; acquisition time = 5.19 minutes; flip angle ($\alpha$) = 78°and SENSE factor (R) = 1.8. During resting state data acquisition subjects were instructed to keep their eyes closed and to let their minds wandering.

A diffusion-weighted spin echo echo-planar imaging (EPI) sequence was used to obtain diffusion-weighted scans with a measured and reconstructed spatial resolution of 2.0 x 2.0 x 2.0 $mm^3$ (acquisition and reconstruction matrix 112 x 112 pixels, 75 slices). Further imaging parameters were: FOV = 224 x 224 $mm^2$; TE = 64.90 ms; repetition time = 18.714 s; $\alpha$ = 90°; SENSE factor R = 2.1; b-value b = 1,000 s/$mm^2$; and number of averages = 1, acquisition time 23:05 minutes. Diffusion was measured along 64 non-collinear directions preceded by a non-diffusion-weighted volume (reference volume).

A T1-weighted fast field echo (FFE) sequence was used to map the $B_0$ field in order to correct the DTI data for EPI-related geometrical distortions. The $B_0$ map (3D echo sequence) is composed of a magnitude and a phase image and was measured with a spatial resolution of 2.0 x 2.0 x 4.0 $mm^3$ (acquisition matrix 112 × 56 pixels, 75 slices) and reconstructed to a spatial resolution of 2.0 x 2.0 x 2.0 $mm^3$ (acquisition matrix 112 × 112 pixels, 75 slices). Further imaging parameters were: FOV = 224 × 224 $mm^2$, 75 slices, dual TE = 3.60 / 5.63 ms, TR = 30.0 ms, $\alpha$ = 60°, acquisition time (min) 4:11.

A volumetric 3D T1-weighted gradient echo sequence (turbo field echo) image was measured with a spatial resolution of 1.0 × 1.0 × 1.0 $mm^3$ (acquisition matrix 240 × 240 pixels, 160 sagittal slices) and reconstructed to a spatial resolution of 0.94 × 0.94 × 1.0 $mm^3$ (reconstruction matrix 256 × 256 pixels, 160 sagittal slices). Further imaging parameters were: FOV = 240 × 240 $mm^2$, slice thickness = 1 mm, number of slices = 160, TE = 3.70 ms, TR = 8.14 ms, $\alpha$ = 8°, SENSE factor R = 1.8, acquisition time 7:29 minutes.

## Brain network construction

We used the popular automated anatomical labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002) in order to define the nodes of the functional and structural brain connectome. We used the AAL version with 90 cortical and subcortical anatomical regions (45 regions of

interest for each hemisphere) excluding the cerebellum.

## Preprocessing of resting-state fMRI data and construction of the functional connectivity network

Functional resting-state MRI data were preprocessed with Data Processing Assistant for Resting-State fMRI (DPARSFA) toolbox version 3.1 (Chao-Gan and Yu-Feng, 2010) set within the Data Processing & Analysis of Brain Imaging (DPABI) toolbox of SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).

Data preprocessing of functional connectivity included the following steps: 1) slice timing correction (mid-slice was used as reference slice); 2) realignment and extraction of head motion parameters according the approach proposed by Power and colleagues (Power et al., 2012; Power et al., 2015); 3) nuisance covariates regression: Friston-24-parameter model. In addition, the global mean, white matter and the cerebrospinal fluid signal were also removed to reduce the effects of non-neuronal BOLD fluctuations; 4) linear- and non-linear spatial normalization to MNI space by using unified segmentation of the T1 image; 5) voxel re-sampling to 2 x 2 x 2 $mm^3$; 6) smoothing with a 4 mm full-width-half-maximum (FWHM) Gaussian kernel; 7) removal of very low (< 0.01 Hz) and high frequencies band (> 0.1 Hz) reported to be of physiological importance (Biswal et al., 1995; Cordes et al., 2001) and 8) time courses were obtained for all regions of interests and then correlated (Pearson) in a region-wise (AAL) manner to generate functional connectivity matrices. To improve the normality of the distribution, these connectivity maps were then converted into z-score maps by Fisher's r-to-z-transformation (Chao-Gan and Yu-Feng, 2010). Each cell in the connectivity matrix contained the correlation value of the preprocessed time series between the ith and the jth node (Zalesky et al., 2010). Finally, these steps resulted in an undirected, weighted 90 x 90 nodes connectivity matrix for each subject.

## Preprocessing of DTI data and construction of the structural connectivity network

Preprocessing of the diffusion-weighted MRI data was performed with FSL tools (FMRIB software library; version 5.0.6; http://www.fmrib.ox.ac.uk/fsl/) (Smith et al., 2004) such as the FDT (FMRIB diffusion toolbox; version 3.0, default parameters) (Behrens et al., 2003). For deterministic fibre tractography we used the Diffusion Toolkit (DTK, version 0.6.2.1) and TrackVis software (version 0.5.2.1; http://trackvis.org/) (Park et al., 2009). The connectivity matrix was computed in MATLAB (version 8.0.0.783; http://www.mathworks.com/index.html).

To construct the connectivity matrix of the WM pathways, the following fully automated preprocessing steps were realized: 1) In a first step, a binary brain mask was created using FSL's brain extraction tool (BET). This mask is used in later steps to exclude non-brain tissue. 2) Eddy current distortions and head movements were corrected using the EDDY_CORRECT tool of FDT. In addition, we performed separate analysis using the tool "eddy" (without the supplementary combination "topup" since we did not acquire b0 images with reverse phase encoding directions) and included the average (after z-score transformations) of the mean of the translational and rotational motion estimation parameters (average volume-by-volume translations and average volume-by-volume rotations) as a nuisance regressor in the analysis with NBS. We included the average of the mean translational and rotational motion estimation parameters as only one nuisance regressor in order to preserve one degree of freedom. 3) EPI-related geometrical distortions due to magnetic field inhomogeneities were unwarped using the $B_0$ map and FSL's FUGUE tool. 4) Diffusion gradients were adjusted for rotations introduced by the eddy current and head movement corrections. 5) The preprocessed DTI data were then subjected to the DTK to compute voxel-wise diffusion tensors and to construct the (principal) eigenvector and eigenvalue maps as well as a map of fractional anisotropy (FA). 6) Deterministic tractography was conducted in DTK using the "brute force" approach with an interpolated streamline tracking algorithm. Twenty streamlines per voxel were propagated and fibre tracking was stopped if FA was lower than 0.10 or if the turning angle of a streamline between two consecutive voxels was larger than 45°. This resulted in a whole brain connectome comprised by about 2-3 millions of streamlines including subcortical pathways and connections to the cerebellum. 7) The individual FA map was registered onto the FMRIB58-FA template, which is in correspondence with the MNI152 standard space, using FSL's linear image registration tool (FLIRT) and the resulting transformations were stored. Due to the nature of the streamlines we run FLIRT and not FNIRT. Applying nonlinear transformations can result in „tearing" streamlines (and in addition, longer streamlines are more prone to tear) so that when computing the connectivity measure (number of streamlines between two brain regions in our study) „teared" streamlines are not counted any longer. 8) These transformations were then applied to the streamlines produced in step 6 in order to transform the streamlines into the MNI152 space. It is important to note that spatial normalization into standard space has been applied after tractography had been performed in native space, i.e. only the resulting streamlines (reconstructed white matter fibres) were transformed, not the preprocessed DTI images that are used for tractography. 9) The automated anatomical labeling (AAL) regions of interest (ROIs) (Tzourio-Mazoyer et al., 2002), which

are already in MNI152 standard space, were used to count the number of streamlines between each pair of ROIs. This AAL template consists of 90 ROIs (45 in each hemisphere) covering the entire neocortex (78 cortical ROIs) as well as the subcortical structures amygdala, hippocampus, thalamus, caudate, putamen, and pallidum (12 subcortical ROIs). 10) Streamlines connected to the cerebellum, those running through the brainstem, and streamlines shorter than 5 mm in length were removed (denoted streamlines omitted). Streamlines that make connections within a ROI itself were deleted (denoted selfloops). The number of the remaining streamlines between any pair of ROIs (denoted streamlines used to populate matrix) was counted using MATLAB scripts (Zalesky et al., 2010). 11) This procedure resulted in an undirected, weighted 90 x 90 nodes (45 nodes per hemisphere) connectivity matrix for each individual participant. The strength of a structural connection was operationalized by the number of reconstructed streamlines between two ROIs. 12) The undirected, weighted 90 x 90 nodes connectivity matrices were then subjected to a network-based statistical analysis (NBS) (Zalesky et al., 2010).

In the case of any structural subnetwork showing group differences or interactions between group and time in number of streamlines, mean fractional anisotropy (FA) values were calculated for each streamline and then also tested in network-based statistics to explore the linkage between the more traditional measure of FA and the network-based mapping measure used in the present study, i.e. the number of reconstructed streamlines.

## A.2 Supplementary Results

### Acute and longitudinal alterations in fractional anisotropy

The increase in number of streamlines in the mTBI group corresponded to a significant increase in FA in 35 out of the 53 investigated edges (Cohen's d = -1.56, CI = -2.012 to -1.108, $p < 0.001$, Supplementary Fig. A.1 and Supplementary Table A.3). Furthermore, increased FA in this 35-edge subnetwork was positively correlated with the number of streamlines within the 53-edge subnetwork ($r = 0.419$, $p = 0.003$). The group x time interaction analysis of FA yielded a subnetwork of 10 edges distributed over 11 nodes indicating a weak trend towards decreased mean FA for the patients at Visit 2 (Cohen's d = -0.6, CI = -1.005 to -0.195, $p = 0.119$, Supplementary Fig. A.2 and Supplementary Table A.5). Within the patients group, the longitudinal FA change of this subnetwork was furthermore positively correlated with the longitudinal alterations of the 19 edge-structural connectivity subnetwork, rather unexpectedly since they displayed a similar topographical pattern ($r = 0.280$, $p = 0.026$, one-sided).

### Comparison between correction methods for eddy current-induced distortions

The whole-brain group comparison analysis in the acute phase using "eddy" together with head motion correction yielded qualitatively comparable results as those obtained when using eddy_correct (Supplementary Fig. A.3–4). Selecting approximately the same set t-threshold of 1.8 the size of the obtained subnetwork (encompassing now 51 nodes) and the direction of the group difference (showing again an increase in structural connectivity in patients compared to healthy controls) were conserved. Common nodes between the two subnetworks were 41, 22 in the left and 19 in the right hemisphere.

### Acute alterations in global efficiency and normalized characteristic path length

Aspects of segregation (clustering, modularity), integration (characteristic path length, global efficiency) and centrality (degree, betweenness centrality) are clearly different from strength of connectivity calculated by using the number of reconstructed streamlines between any two nodes. It seems intuitive that a direct comparison is not feasible. Nevertheless, to dissolve the apparent contradiction, we run new analyses examining the entire connectivity matrix at Visit 1 but adopting the graph theoretical measure "global efficiency" (average inverse shortest path length) and "normalized characteristic path length" (characteristic path length of the network normalized to an appropriate null network). Consistently with the literature, there was a decrease in global efficiency and an increase in normalized characteristic path length in mTBI patients compared with healthy controls (Caeyenberghs et al., 2012; Caeyenberghs et al., 2014; Yuan et al., 2014). Both network-level measures of structural connectivity did not however reach a statistical significance ($p > 0.409$). This was probably due to the fact that we explored group-wise differences at large-scale instead of within "our 53-edge structural subnetwork" that demonstrated increased connectivity in the acute phase.

## A.3 Supplementary Tables and Figures

**Supplementary Table A.1 Neuropsychological assessment scores (uncorrected and adjusted p-values for multiple comparisons using false discovery rate)**

| | Visit 1 (acute phase) Group differences (unpaired t-test) | | Visit 2 (chronic phase) Group differences (unpaired t-test) | | Changes within groups over time (paired t-test) | | | |
|---|---|---|---|---|---|---|---|---|
| | p-value (uncorrected) | p-value (adjusted for FDR) | p-value (uncorrected) | p-value (adjusted for FDR) | Patients (uncorrected p-value) | Patients (adjusted for FDR) | Controls (uncorrected p-value) | Controls (adjusted for FDR) |
| **Neuropsychological assessment** | | | | | | | | |
| RPQ (total score) | <0.001 | 0.006 | 0.005 | 0.019 | <0.001 | 0.006 | 0.537 | 0.612 |
| Alertness, tonic (ms) | 0.007 | 0.021 | 0.593 | 0.660 | 0.004 | 0.018 | 0.156 | 0.232 |
| Alertness, phasic (ms) | 0.031 | 0.065 | 0.992 | 0.992 | 0.005 | 0.019 | 0.098 | 0.160 |
| Go/Nogo (ms) | 0.019 | 0.047 | 0.059 | 0.100 | 0.019 | 0.047 | 0.042 | 0.081 |
| Go/Nogo (errors) | 0.606 | 0.660 | 0.828 | 0.845 | 0.726 | 0.757 | 0.453 | 0.555 |
| Divided attention, auditory (ms) | 0.145 | 0.222 | 0.134 | 0.212 | 0.025 | 0.058 | 0.013 | 0.038 |
| Divided attention, visual (ms) | 0.014 | 0.038 | 0.046 | 0.084 | <0.001 | 0.006 | <0.001 | 0.006 |
| Working memory | 0.050 | 0.088 | 0.641 | 0.683 | 0.001 | 0.006 | 0.391 | 0.498 |
| AVLGT recall score | 0.178 | 0.249 | 0.326 | 0.432 | <0.001 | 0.006 | <0.001 | 0.006 |
| AVLGT long delay | 0.476 | 0.560 | 0.480 | 0.560 | 0.002 | 0.011 | <0.001 | 0.006 |
| BDI-II (score) | 0.006 | 0.021 | 0.167 | 0.241 | 0.003 | 0.015 | 0.275 | 0.374 |
| BAI (score) | 0.032 | 0.065 | 0.007 | 0.021 | 0.396 | 0.498 | 0.043 | 0.081 |
| Intellectual ability (IQ) | 0.031 | 0.065 | - | - | - | - | - | - |

*Note:* The adjustment for false discovery rate occurred over all 49 tests (https://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/).
AVLGT = German adaptation of the Rey Auditory Verbal Learning Tests RAVLT; BAI = Beck Anxiety Inventar; BDI-II = Beck Depression Inventory, 2nd edition; FDR = false discovery rate; ms = ms milliseconds; RPQ = Rivermead Post-Concussion Symptoms Questionnaire.

**Supplementary Table A.2 Reduced resting-state functional connectivity in a 15-edge subnetwork for patients compared to controls at Visit 1**

| List of functional connections | | |
|---|---|---|
| | | t-value |
| Precuneus_L | Heschl_L | 3.87 |
| Heschl_R | Temporal_Pole_Sup_L | 3.81 |
| Cingulum_Ant_R | ParaHippocampal_R | 3.77 |
| Cingulum_Ant_L | ParaHippocampal_R | 3.61 |
| Cingulum_Ant_L | Cingulum_Post_R | 3.60 |
| Cingulum_Ant_L | Cingulum_Post_L | 3.52 |
| Precuneus_L | Heschl_R | 3.49 |
| Cingulum_Post_L | Temporal_Sup_R | 3.41 |
| Precuneus_R | Heschl_L | 3.36 |
| Amygdala_R | Heschl_R | 3.36 |
| Temporal_Sup_L | Temporal_Pole_Mid_R | 3.31 |
| Cingulum_Ant_R | Cingulum_Post_L | 3.30 |
| Supp_Motor_Area_R | Cingulum_Ant_L | 3.23 |
| Heschl_R | Temporal_Pole_Mid_R | 3.23 |
| ParaHippocampal_R | Heschl_L | 3.17 |

*Note:* Cohen's d = 1.59, CI = 1.138–2.046, p = 0.0057.
Ant = anterior; L = left; Mid = middle; Post = posterior; R = right; Sup = superior; Supp = supplementary.

**Supplementary Table A.3 Increased DTI-based structural connectivity in a 53-edge subnetwork for patients compared to controls at Visit 1**

| List of structural connections | | | |
|---|---|---|---|
| | | t-value (streamlines) | t-value (FA) |
| Fusiform_L | Temporal_Inf_L | 3.46 | 0.99 |
| Rolandic_Oper_L | Heschl_L | 2.87 | |
| Cingulum_Post_R | Lingual_R | 2.78 | |
| Frontal_Sup_Medial_L | Cingulum_Ant_L | 2.76 | 1.35 |
| Olfactory_R | ParaHippocampal_R | 2.74 | 1.16 |
| Rectus_L | Pallidum_L | 2.48 | 0.02 |
| Insula_R | Temporal_Pole_Sup_R | 2.48 | |
| Precentral_L | Thalamus_L | 2.46 | 1.41 |
| Supp_Motor_Area_R | Frontal_Sup_Medial_R | 2.43 | 0.94 |
| Fusiform_R | Temporal_Mid_R | 2.41 | 0.19 |
| Caudate_L | Putamen_R | 2.32 | 0.15 |
| ParaHippocampal_R | Temporal_Mid_R | 2.3 | 2.33 |
| Insula_L | Temporal_Pole_Mid_L | 2.28 | 3.17 |
| Putamen_L | Putamen_R | 2.28 | 1.15 |
| Precuneus_R | Thalamus_R | 2.27 | 1.90 |
| Calcarine_L | Precuneus_R | 2.23 | 0.63 |
| Frontal_Mid_R | Putamen_R | 2.23 | |
| Frontal_Sup_Medial_R | Putamen_R | 2.21 | 0.88 |
| Occipital_Mid_L | Temporal_Mid_L | 2.2 | 0.29 |
| Putamen_L | Pallidum_L | 2.19 | 0.03 |
| Postcentral_L | Hippocampus_R | 2.19 | 2.81 |
| Postcentral_L | Putamen_L | 2.18 | 3.21 |
| Supp_Motor_Area_L | Thalamus_L | 2.17 | 1.76 |
| Supp_Motor_Area_L | Precuneus_L | 2.16 | 1.71 |
| Cingulum_Post_R | Fusiform_R | 2.16 | 2.38 |
| Amygdala_R | Thalamus_R | 2.16 | 1.85 |
| Insula_R | ParaHippocampal_R | 2.14 | |
| Frontal_Sup_Orb_R | Cingulum_Ant_R | 2.11 | |
| Cingulum_Post_L | Precuneus_L | 2.1 | |
| Calcarine_R | Lingual_R | 2.1 | |
| Putamen_L | Frontal_Med_Orb_R | 2.03 | 2.72 |
| Frontal_Sup_R | Cingulum_Post_R | 2.02 | |
| Parietal_Sup_R | Temporal_Mid_R | 2.02 | 0.33 |
| Frontal_Mid_Orb_L | Supp_Motor_Area_L | 2.01 | 1.19 |
| Frontal_Mid_Orb_R | Cingulum_Ant_R | 2.01 | |
| Supp_Motor_Area_L | Olfactory_L | 1.99 | 2.86 |
| Thalamus_L | Temporal_Mid_L | 1.99 | 1.31 |
| Insula_R | Temporal_Sup_R | 1.98 | |
| Frontal_Sup_R | Frontal_Sup_Orb_R | 1.97 | |
| Olfactory_R | Caudate_R | 1.97 | |
| Fusiform_R | Precuneus_R | 1.96 | 2.08 |

| | | | |
|---|---|---|---|
| Frontal_Sup_Orb_L | Putamen_L | 1.95 | 0.01 |
| Rolandic_Oper_L | Temporal_Pole_Sup_L | 1.95 | |
| Rectus_L | Insula_L | 1.94 | 0.95 |
| Fusiform_L | Temporal_Mid_L | 1.93 | 2.25 |
| Frontal_Sup_Orb_L | Cingulum_Ant_L | 1.92 | |
| Supp_Motor_Area_L | Amygdala_L | 1.92 | 0.79 |
| Occipital_Mid_R | Temporal_Mid_R | 1.91 | |
| Precentral_L | Cingulum_Ant_L | 1.88 | 1.71 |
| Amygdala_L | Pallidum_L | 1.88 | 0.79 |
| Parietal_Sup_R | Temporal_Sup_R | 1.88 | |
| Hippocampus_R | Temporal_Mid_R | 1.88 | 0.73 |
| Insula_L | Temporal_Pole_Sup_L | 1.87 | |

*Note:*
Analysis with number of streamlines: Cohen's d = -1.71, CI = -2.168 to -1.243, p = 0.041
Analysis with fractional anisotropy-value: Cohen's d = -1.56, CI = -2.012 to -1.108, p < 0.001
Ant = anterior; FA = fractional anisotropy; Inf = inferior; L = left; Med = medialis; Mid = middle; Oper = operculum; Orb = orbitalis; Post = posterior; R = right; Sup = superior; Supp = supplementary.

**Supplementary Table A.4 Functional connectivity: selective interaction within the 15-edge subnetwork of interest resulting from group comparison at Visit 1**

| List of functional connections | | |
|---|---|---|
| | | t-value |
| Cingulum_Post_L | Temporal_Sup_R | 3.33 |
| Temporal_Sup_L | Temporal_Pole_Mid_R | 3.02 |
| Cingulum_Ant_L | ParaHippocampal_R | 2.33 |
| Heschl_R | Temporal_Pole_Mid_R | 2.23 |
| Cingulum_Ant_R | ParaHippocampal_R | 2.17 |
| Amygdala_R | Heschl_R | 2.08 |
| Heschl_R | Temporal_Pole_Sup_L | 1.96 |
| Cingulum_Ant_L | Cingulum_Post_L | 1.95 |
| Cingulum_Ant_L | Cingulum_Post_R | 1.82 |
| Cingulum_Ant_R | Cingulum_Post_L | 1.51 |
| ParaHippocampal_R | Heschl_L | 1.1 |
| Precuneus_L | Heschl_L | 1.09 |
| Precuneus_L | Heschl_R | 1.08 |
| Precuneus_R | Heschl_L | 0.99 |
| Supp_Motor_Area_R | Cingulum_Ant_L | 0.93 |

*Note:* Cohen's d = 0.9, CI = 0.490–1.321, p = 0.002.
Ant = anterior; L = left; Mid = middle; Post = posterior; R = right; Sup = superior; Supp = supplementary.

**Supplementary Table A.5 Structural connectivity: selective interaction within the 53-edge subnetwork (streamlines) respectively the 35-edge subnetwork (fractional anisotropy values) of interest resulting from group comparison at Visit 1**

| List of structural connections | | | |
|---|---|---|---|
| Network | | t-value (streamlines) | t-value (FA) |
| 1 | | | |
| Precentral_L | Cingulum_Ant_L | 2.21 | 0.92 |
| Putamen_L | Frontal_Med_Orb_R | 2.16 | |
| Frontal_Sup_Orb_L | Putamen_L | 2.07 | |
| Supp_Motor_Area_R | Frontal_Sup_Medial_R | 1.69 | |
| Frontal_Sup_Medial_L | Cingulum_Ant_L | 1.44 | 0.1 |
| Putamen_L | Pallidum_L | 1.24 | |
| Caudate_L | Putamen_R | 1.19 | |
| Supp_Motor_Area_L | Amygdala_L | 1.06 | |
| Putamen_L | Putamen_R | 0.77 | |
| Frontal_Mid_Orb_L | Supp_Motor_Area_L | 0.69 | 1.88 |
| Postcentral_L | Putamen_L | 0.62 | |
| Precentral_L | Thalamus_L | 0.55 | 0.13 |
| Frontal_Sup_Orb_L | Cingulum_Ant_L | 0.5 | |
| Supp_Motor_Area_L | Olfactory_L | 0.41 | 0.17 |
| Supp_Motor_Area_L | Thalamus_L | 0.32 | 1.3 |
| Amygdala_L | Pallidum_L | 0.22 | |
| Supp_Motor_Area_L | Precuneus_L | 0.19 | 0.15 |
| Frontal_Mid_R | Putamen_R | 0.14 | |
| Frontal_Sup_Medial_R | Putamen_R | 0.03 | |
| 2 | | | |
| Calcarine_L | Precuneus_R | 2.19 | |
| Frontal_Sup_Orb_R | Cingulum_Ant_R | 2.04 | |
| Amygdala_R | Thalamus_R | 1.93 | |
| Hippocampus_R | Temporal_Mid_R | 1.85 | |
| Cingulum_Post_R | Fusiform_R | 1.83 | |
| Precuneus_R | Thalamus_R | 1.77 | |
| ParaHippocampal_R | Temporal_Mid_R | 1.75 | |
| Parietal_Sup_R | Temporal_Sup_R | 1.7 | |
| Frontal_Sup_R | Frontal_Sup_Orb_R | 1.67 | |
| Occipital_Mid_R | Temporal_Mid_R | 1.52 | |
| Fusiform_R | Precuneus_R | 1.43 | |
| Frontal_Sup_R | Cingulum_Post_R | 1.25 | |
| Olfactory_R | ParaHippocampal_R | 1.2 | |
| Parietal_Sup_R | Temporal_Mid_R | 0.9 | |
| Frontal_Mid_Orb_R | Cingulum_Ant_R | 0.89 | |
| Cingulum_Post_R | Lingual_R | 0.66 | |
| Calcarine_R | Lingual_R | 0.57 | |
| Fusiform_R | Temporal_Mid_R | 0.34 | |

*Note:*
Analysis with number of streamlines: Subnetwork 1: Cohen's d = -0.72, CI = -1.132– -0.315, p = 0.025. Subnetwork 2: Cohen's d = -0.71, CI = -1.120– -0.303, p = 0.035.
Analysis with fractional anisotropy-value: Cohen's d = -0.6, CI = -1.005– -0.195, p = 0.119.
In addition to the edges listed above, the interaction analysis revealed also three more connections: Fusiform_L to Temporal_Mid_L = 2.19, Thalamus_L to Temporal_Mid_L = 0.44, Fusiform_L to Temporal_Inf_L = 0.18.
Ant = anterior; FA = fractional anisotropy; Inf = inferior; L = left; Med = medialis; Mid = middle; Orb = orbitalis; Post = posterior; R = right; Sup = superior; Supp = supplementary.

**Supplementary Table A.6 Functional connectivity: whole-brain group x time interaction in subnetwork of 59 edges and 48 nodes**

| List of functional connections | | |
|---|---|---|
| | | t-value |
| Occipital_Mid_R | Temporal_Pole_Sup_L | 4.01 |
| Frontal_Med_Orb_L | Cingulum_Mid_L | 3.91 |
| Paracentral_Lobule_L | Thalamus_L | 3.53 |
| Frontal_Med_Orb_R | Cingulum_Mid_L | 3.5 |
| Frontal_Sup_Medial_R | Temporal_Sup_R | 3.48 |
| Frontal_Sup_Orb_L | Angular_R | 3.35 |
| Occipital_Sup_R | Temporal_Pole_Sup_L | 3.35 |
| Cingulum_Post_L | Temporal_Sup_R | 3.33 |
| Cuneus_L | Temporal_Pole_Sup_L | 3.3 |
| Olfactory_R | Calcarine_L | 3.14 |
| Cuneus_R | Temporal_Pole_Sup_L | 3.14 |
| Occipital_Sup_R | Occipital_Mid_R | 3.1 |
| Frontal_Med_Orb_R | Putamen_L | 3.08 |
| Cingulum_Post_L | Caudate_R | 3.03 |
| Occipital_Mid_R | Temporal_Pole_Mid_L | 3.02 |
| Temporal_Sup_L | Temporal_Pole_Mid_R | 3.02 |
| Frontal_Mid_Orb_R | Frontal_Sup_Medial_R | 2.97 |
| Frontal_Med_Orb_R | Cingulum_Mid_R | 2.97 |
| Frontal_Inf_Orb_L | Cuneus_L | 2.95 |
| Thalamus_R | Temporal_Mid_L | 2.92 |
| Frontal_Med_Orb_L | Temporal_Sup_R | 2.89 |
| Frontal_Inf_Orb_L | Occipital_Mid_R | 2.85 |
| Olfactory_L | Cingulum_Mid_R | 2.78 |
| Frontal_Med_Orb_R | Putamen_R | 2.77 |
| Cuneus_R | Thalamus_L | 2.77 |
| Frontal_Med_Orb_L | SupraMarginal_R | 2.76 |
| Frontal_Med_Orb_L | Putamen_L | 2.76 |
| Cingulum_Post_L | Temporal_Pole_Sup_R | 2.76 |
| ParaHippocampal_L | Cuneus_L | 2.74 |
| Thalamus_R | Temporal_Sup_L | 2.73 |
| Frontal_Med_Orb_R | Pallidum_R | 2.71 |
| Hippocampus_L | Occipital_Sup_R | 2.7 |
| Frontal_Sup_Medial_L | Temporal_Sup_R | 2.69 |
| Frontal_Inf_Orb_L | Cuneus_R | 2.66 |
| Insula_L | Temporal_Pole_Mid_R | 2.62 |
| Angular_R | Temporal_Pole_Sup_L | 2.59 |
| Olfactory_L | Calcarine_L | 2.58 |
| Precuneus_L | Thalamus_L | 2.58 |
| Insula_R | Temporal_Pole_Mid_R | 2.58 |
| Frontal_Med_Orb_R | Temporal_Sup_R | 2.57 |
| Frontal_Inf_Oper_R | Olfactory_L | 2.55 |
| Supp_Motor_Area_L | Heschl_R | 2.54 |

| | | |
|---|---|---|
| Cuneus_L | Temporal_Pole_Sup_R | 2.53 |
| Putamen_L | Pallidum_L | 2.52 |
| Rectus_R | Occipital_Mid_R | 2.51 |
| Frontal_Mid_Orb_L | Angular_R | 2.51 |
| Frontal_Sup_Medial_R | Heschl_R | 2.51 |
| Frontal_Med_Orb_L | Cingulum_Mid_R | 2.5 |
| ParaHippocampal_L | Occipital_Sup_L | 2.5 |
| Frontal_Sup_Orb_L | Occipital_Mid_R | 2.46 |
| Paracentral_Lobule_L | Temporal_Sup_L | 2.46 |
| Frontal_Med_Orb_L | Pallidum_R | 2.45 |
| Frontal_Inf_Oper_L | Angular_R | 2.44 |
| Cingulum_Mid_L | Temporal_Pole_Sup_L | 2.44 |
| Occipital_Sup_L | Temporal_Pole_Sup_L | 2.44 |
| Rolandic_Oper_L | Paracentral_Lobule_L | 2.43 |
| ParaHippocampal_R | Pallidum_L | 2.43 |
| Cuneus_L | Thalamus_L | 2.43 |
| Occipital_Sup_R | Temporal_Pole_Mid_R | 2.42 |

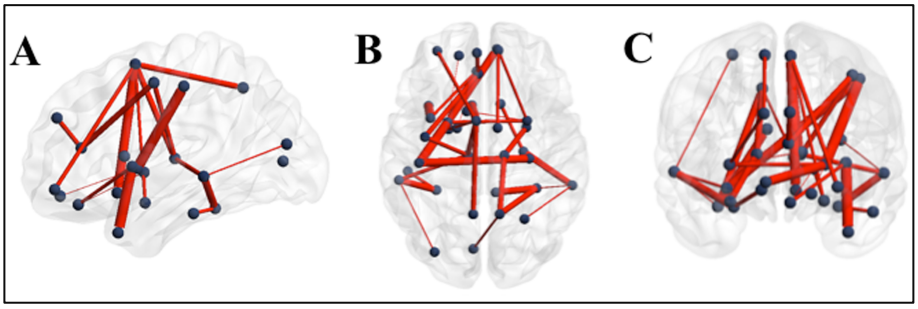
*Note:* Cohen's d = 1.87, CI = 1.402–2.353, p = 0.045.
Inf = inferior; L = left; Med = medialis; Mid = middle; Oper = operculum; Orb = orbitalis; Post = posterior; R = right; Sup = superior; Supp = supplementary.

**Supplementary Table A.7 Spearman rank-order correlations between changes in mean connectivity (functional and structural) and in cognitive performance across time points**

| | mTBI patients (*n* = 49) | | |
|---|---|---|---|
| | **Functional subnetwork (15 edges)** | **Structural subnetwork 1 (19 edges)** | **Structural subnetwork 2 (18 edges)** |
| Alertness, tonic (RT) | rho = 0.044<br>p = 0.385 | rho = 0.098<br>p = 0.257 | rho = -0.070<br>p = 0.323 |
| Alertness, phasic (RT) | rho = 0.036<br>p = 0.405 | rho = 0.092<br>p = 0.271 | rho = 0.075<br>p = 0.310 |
| Go/Nogo (RT) | rho = -0.171<br>p = 0.128 | rho = 0.010<br>p = 0.473 | rho = -0.114<br>p = 0.225 |
| Divided attention auditory (RT) | rho = 0.053<br>p = 0.364 | rho = -0.083<br>p = 0.292 | rho = -0.262<br>p uncorrected = 0.039<br>p corrected = 0.264 |
| Divided attention visual (RT) | rho = 0.333<br>p uncorrected = 0.012<br>p corrected = 0.120 | rho = 0.089<br>p = 0.278 | rho = -0.038<br>p = 0.401 |
| Working memory | rho = -0.350<br>p uncorrected = 0.008<br>p corrected = 0.120 | rho = -0.168<br>p = 0.133 | rho = -0.101<br>p = 0.252 |
| Recall score (AVLGT) | rho = -0.044<br>p = 0.385 | rho = 0.348<br>p uncorrected = 0.009<br>p corrected = 0.120 | rho = -0.194<br>p = 0.098 |
| Long delay (AVLGT) | rho = -0.183<br>p = 0.112 | rho = 0.091<br>p = 0.273 | rho = -0.126<br>p = 0.201 |
| BDI-II | rho = 0.108<br>p = 0.236 | rho = 0.254<br>p uncorrected = 0.0445<br>p corrected = 0.264 | rho = 0.019<br>p = 0.449 |
| BAI | rho = 0.054<br>p = 0.360 | rho = -0.097<br>p = 0.260 | rho = 0.111<br>p = 0.231 |

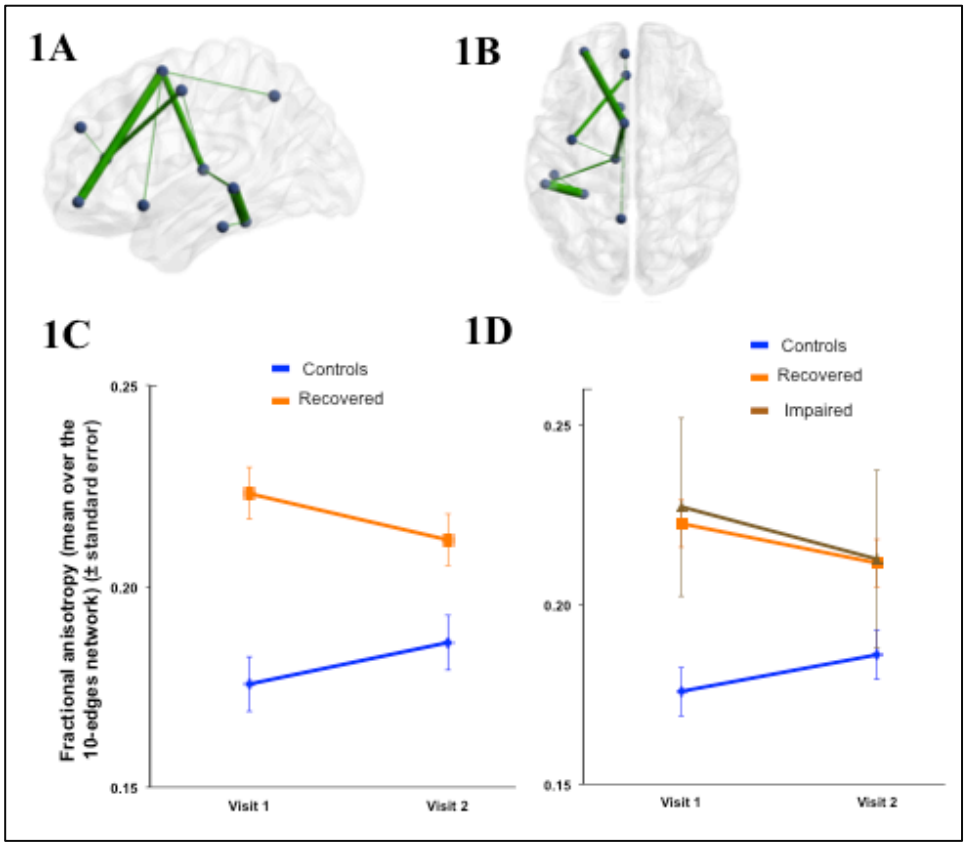
*Note:* The adjustment for false discovery rate (FDR) occurred over all 49 tests (https://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/). P-values are reported one-tailed. Rho = partial Spearman's rank-order correlation.
AVLGT = German adaptation of the Rey Auditory Verbal Learning Tests RAVLT; BAI = Beck Anxiety Inventar; BDI-II = Beck Depression Inventory, 2nd edition; RT = reaction time.

**Supplementary Figure A.1 Increased fractional anisotropy in a 35-edge subnetwork deriving from the 53-edge subnetwork for patients compared to controls at Visit 1**



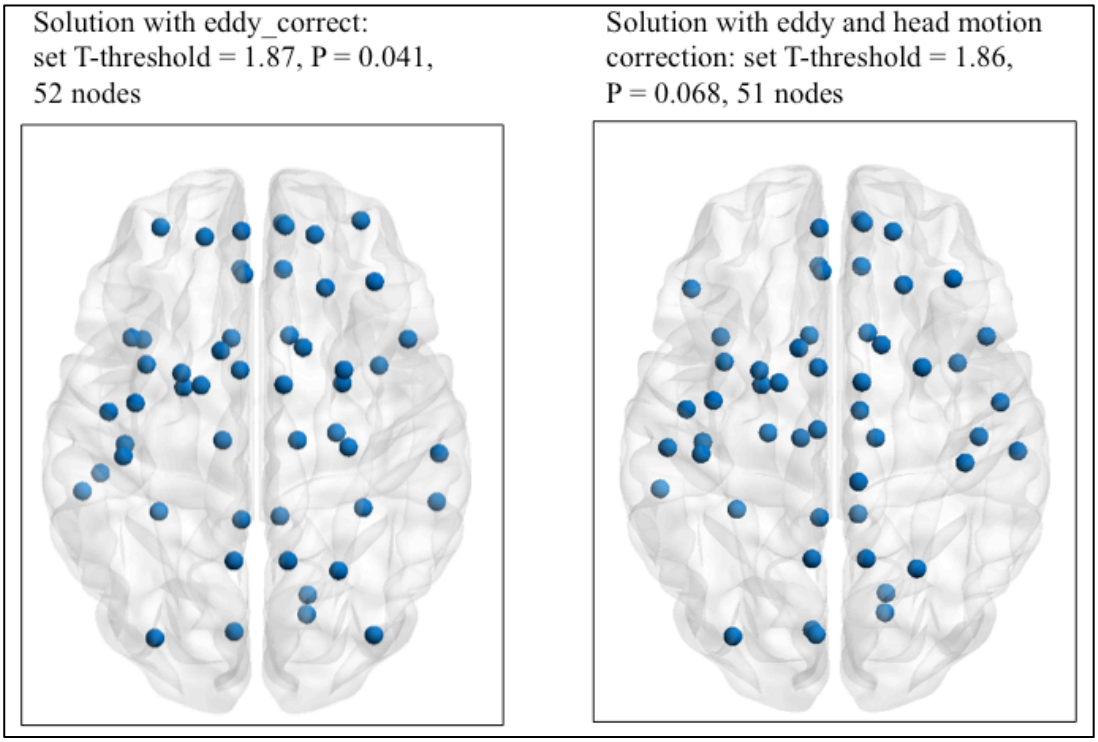

*Note:* The NBS-specific set threshold was set to t = 0 in order to admit all possible connections of the 53-edge subnetwork to the set of suprathreshold links showing a change over time.
Cohen's d = -1.56, CI = -2.012– 1.108, p < 0.001. A = left, B = top, C = frontal.

**Supplementary Figure A.2 Changes in fractional anisotropy within the initially impaired 35-edge subnetwork over 1 year resulted in a 10-edge subnetwork (selective group x time interaction)**

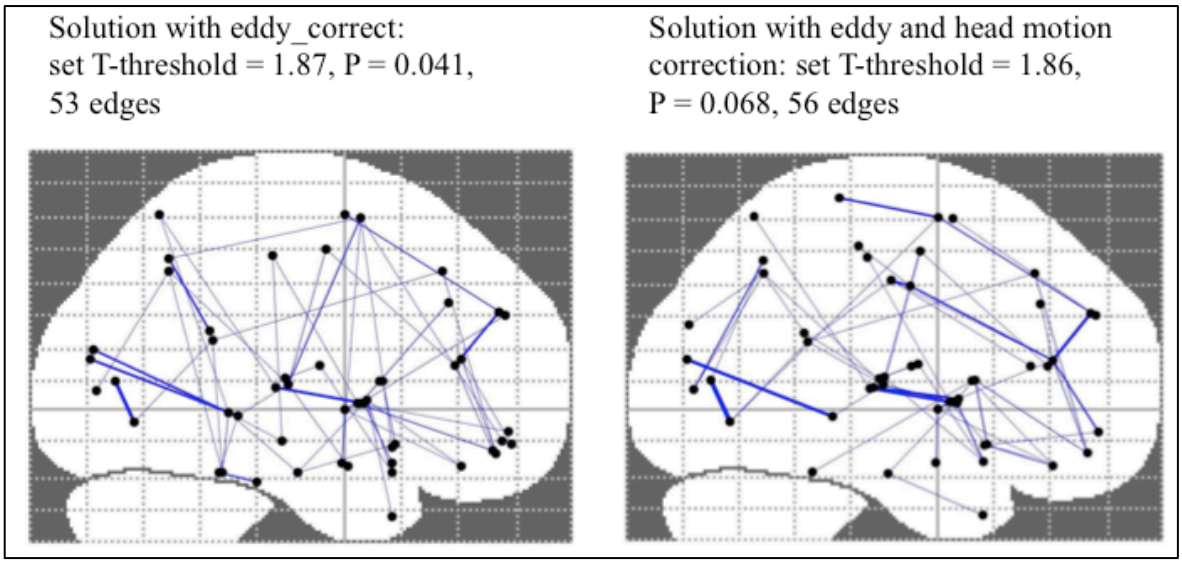



*Note: 1A*= left; *1B* = top; *1C* = A decrease in FA in the patient sample and an increase in the control sample were responsible for the significant repeated-measures effect. The group effect at Visit 1 reached a strong significance ($p < 0.001$) that shifted into a weaker significance at Visit 2 ($p = 0.004$). The time effect for each group revealed trend-wise changes (patients $p = 0.098$, controls $p = 0.152$) over 1 year. *1D* = At a descriptive level, the recovery curves of the subcohorts of patients with and without PCS were similar. The NBS-specific sensitivity threshold was set to $t = 0$ in order to admit all possible connections of the 35-edge subnetwork to the set of suprathreshold links showing a change over time.
Cohen's d = -0.6, CI = -1.005– -0.195, p = 0.119.

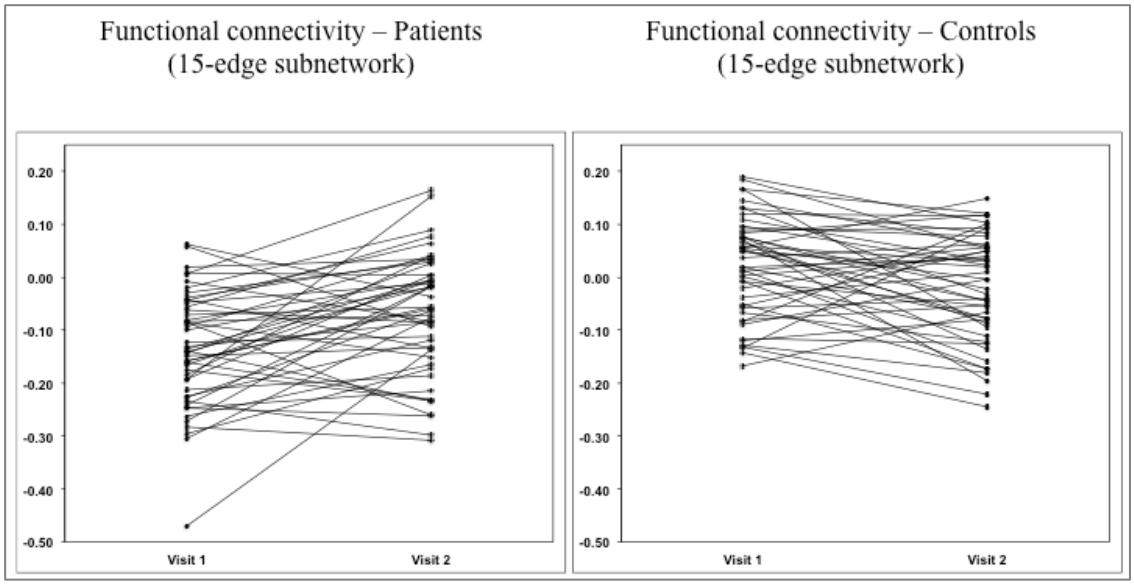
**Supplementary Figure A.3 Comparison between correction efficiency of FSL tools: "eddy_correct" (left) against the combination of "eddy" with head motion estimation as a nuisance regressor at node level**



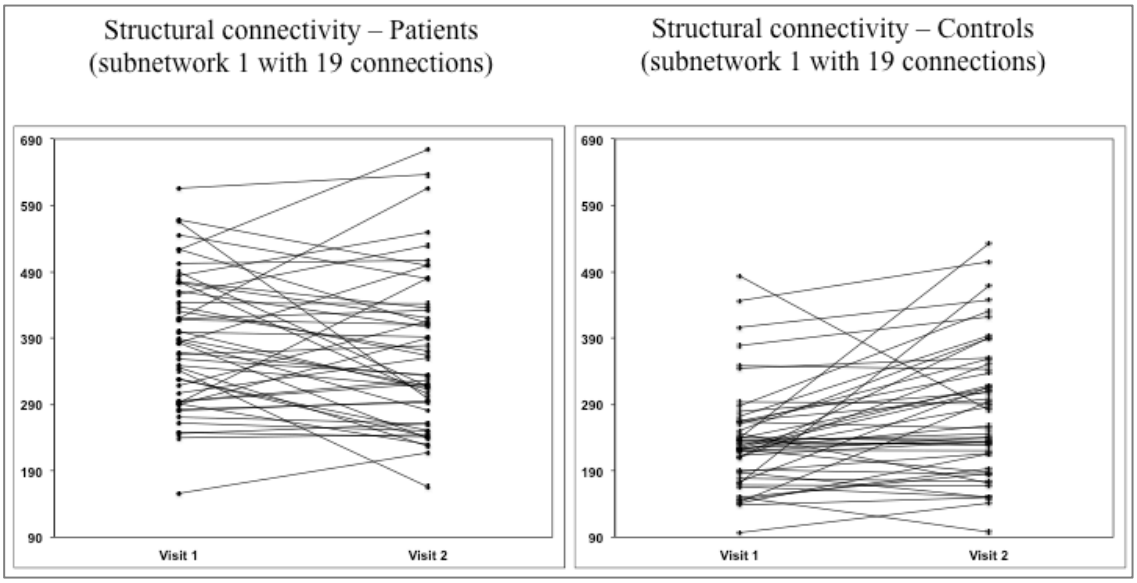

**Supplementary Figure A.4 Comparison between correction efficiency of FSL tools: "eddy_correct" (left) against the combination of "eddy" with head motion estimation as a nuisance regressor at edge level**



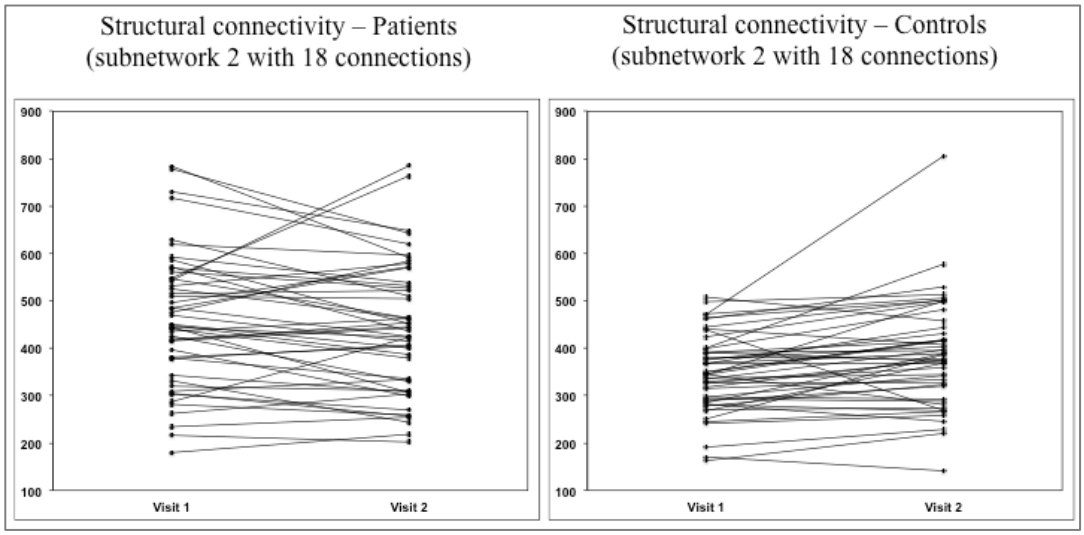
**Supplementary Figure A.5 Functional connectivity changes (derived from the selective interaction analysis) between visits of the 15-edge subnetwork separately for each patient (left) and each control (right)**




**Supplementary Figure A.6 Structural connectivity changes (derived from the selective interaction analysis) between visits of the 19-edge left-hemispheric lateralized subnetwork separately for each patient (left) and each control (right)**



**Supplementary Figure A.7 Structural connectivity changes (derived from the selective interaction analysis) between visits of the 18-edge right-hemispheric lateralized subnetwork separately for each patient (left) and each control (right)**



# B Supplementary material of STUDY II

## B.1 Supplementary Methods

### Neuropsychological assessment

The neuropsychological investigation focusing on the domains of attention, executive and memory functions included the following tests: (i) subtests alertness (tonic and phasic), Go/Nogo (1 from 2), and divided attention of the Test for Attentional Performance (TAP 2.2, Testbatterie für Aufmerksamkeitsprüfung) to measure reaction time, inhibitory control, and cognitive flexibility with varying complexity; (ii) German version (Von Aster et al., 2006) of the backward digit span of the Wechsler Adult Intelligence Scale WAIS-III (Wechsler, 1997) to assess verbal working memory; and (iii) Swiss adaptation (Balzer et al., 2011) of the Rey Auditory Verbal Learning Test RAVLT (Strauss et al., 2006) to assess verbal learning (using the total number of words recalled across five trials) and long-delay verbal recall (after 30 min). Of all tests, only the most relevant parameter is presented to avoid overrepresentation of one test.

In addition, two scales were used to assess emotional symptomatology: the German version (Hautzinger et al., 2006) of the Beck Depression Inventory 2nd edition BDI-II (Beck et al., 1996) was selected to control for manifestations of depression; and the German version (Margraf and Ehlers, 2007) of the Beck Anxiety Inventory BAI (Beck and Steer, 1993) was chosen to evaluate anxiety symptoms in response to mTBI.

## B.2 Supplementary Tables and Figures

**Supplementary Table B.1 Cortical thickness differences between patients and controls at Scan 2 (whole brain approach)**

| Measure and anatomical location (cortical thickness) | Cluster size ($mm^2$) | MNI coordinates | | | CWP |
|---|---|---|---|---|---|
| | | x | y | z | |
| Left cluster: APFC, MPFC | 4141 | -15.2 | 16.6 | -17.4 | 0.0002 |
| Left SOG, MOG | 2082 | -12.5 | -92.4 | 20.2 | 0.0058 |
| Right APFC | 1875 | 31.2 | 49.6 | 3.9 | 0.016 |
| Right SOG, IPS | 3309 | 30.5 | -62.3 | 43.6 | 0.0002 |

*Note:* MNI coordinates = coordinates of the maximum value found in the cluster within the MNI space; CWP = clusterwise-corrected p value. APFC = anterior prefrontal cortex; IPS = sulcus intraparietalis; MOG = middle occipital gyrus; MPFC = medial prefrontal cortex; SOG = superior occipital gyrus.

**Supplementary Table B.2 Partial Spearman correlations between changes in prefrontal cortical thickness and changes in cognitive performance in the mTBI patients between Visit 1 and Visit 2**

| Cognitive tests | mTBI patients (*n* = 49)<br>Mean prefrontal cluster |
|---|---|
| Alertness (RT) | rho = 0.030<br>P = 0.421 |
| Go/Nogo (RT) | rho = -0.236<br>P = 0.057, q = 0.095 |
| Divided attention (RT) | rho = -0.054<br>P = 0.361 |
| Working memory (score) | rho = 0.236<br>P = 0.057, q = 0.095 |
| Verbal memory (score) | rho = 0.302<br>P = 0.021, q = 0.095 |

*Note:* Alertness (mean of tonic and phasic alertness), divided attention (mean of visual and auditory scores), and verbal memory (mean z-score of learning and long delay from the German adaptation of the Rey Auditory Verbal Learning Test) represent *composites*. The adjustment for false discovery rate (FDR) occurred over all five tests (https://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/). None of these results survived the post hoc adjustment with FDR (<0.05) for multiple comparisons.

RT = reaction time.

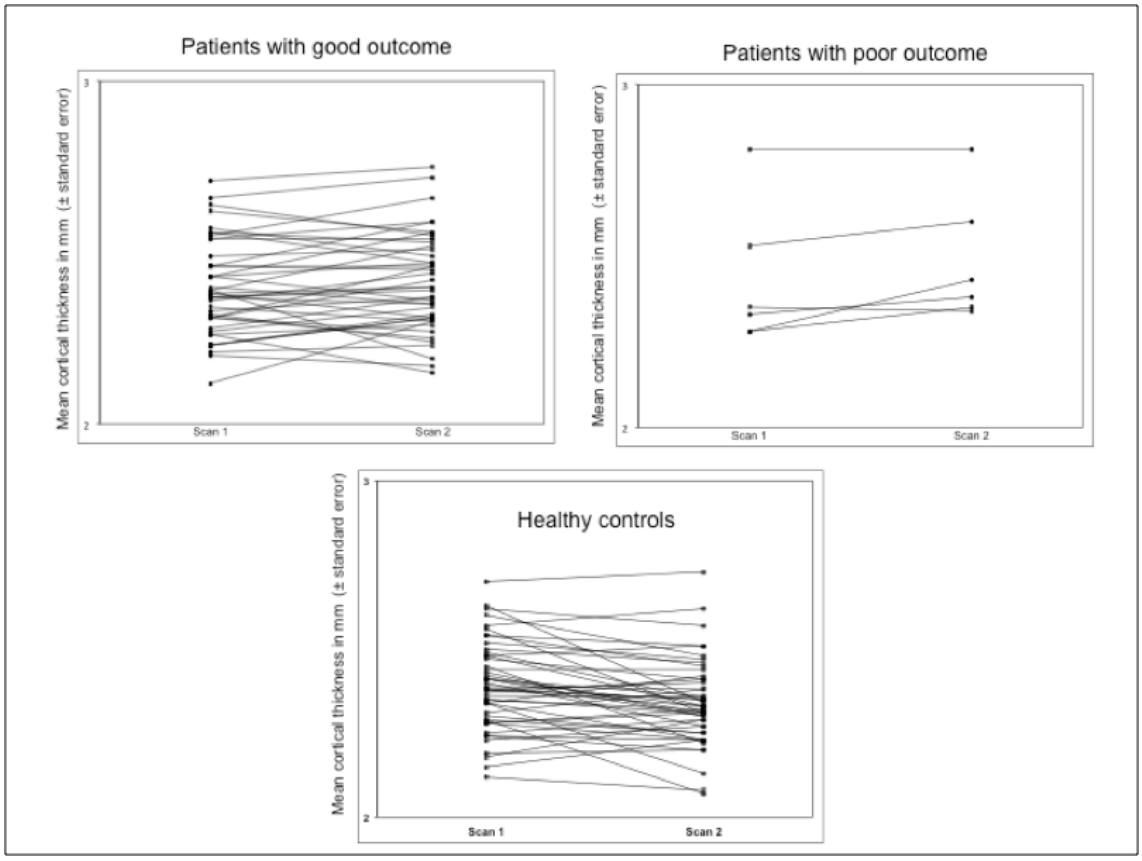
**Supplementary Table B.3 Partial Spearman correlations between changes in prefrontal cortical thickness and changes in cognitive performance in good outcome and poor outcome patients between Visit 1 and Visit 2**

| **Cognitive tests** | **Good outcome (*n* = 43)**<br>**Mean prefrontal cluster** | **Poor outcome (*n* = 6)**<br>**Mean prefrontal cluster** |
|---|---|---|
| Alertness (RT) | rho = -0.085<br>P = 0.302 | rho = 0.831<br>P = 0.188 |
| Go/Nogo (RT) | rho = -0.314<br>P = 0.024, q = 0.090 | rho = -0.992<br>P = 0.039, q = 0.090 |
| Divided attention (RT) | rho = -0.156<br>P = 0.168 | rho = 0.995<br>P = 0.033, q = 0.090 |
| Working memory (score) | rho = 0.272<br>P = 0.045, q = 0.090 | rho = 0.947<br>P = 0.104 |
| Verbal memory (score) | rho = 0.321<br>P = 0.022, q = 0.090 | rho = -0.653<br>P = 0.274 |

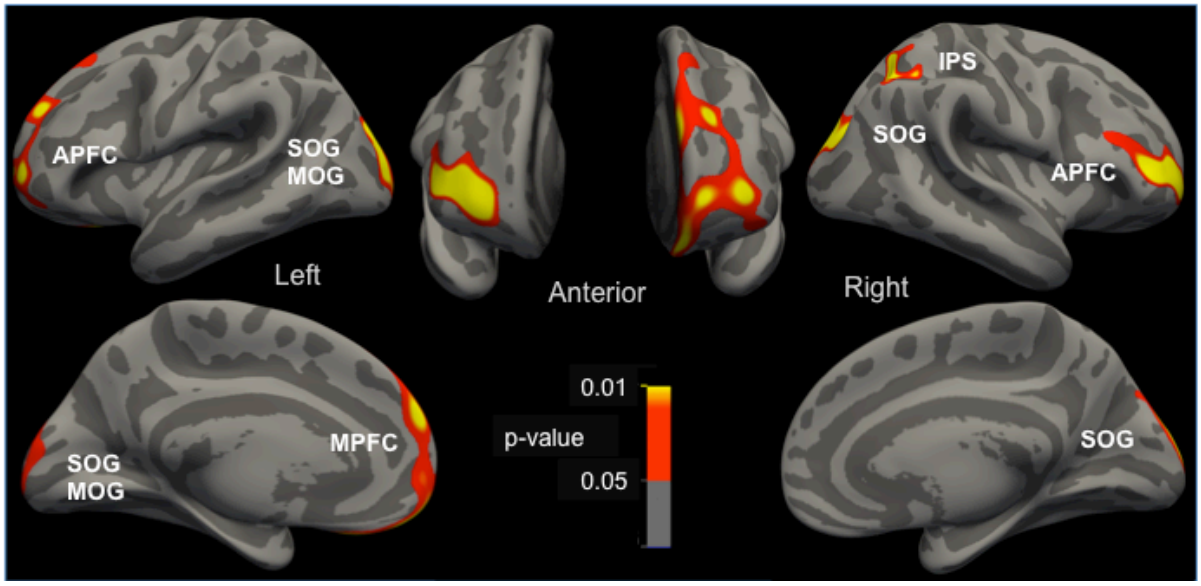
*Note:* Alertness (mean of tonic and phasic alertness), divided attention (mean of visual and auditory scores), and verbal memory (mean z-score of learning and long delay from the German adaptation of the Rey Auditory Verbal Learning Test) represent *composites*. The adjustment for false discovery rate (FDR) occurred over all 10 tests (https://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/). None of these results survived the post hoc adjustment with FDR (< 0.05) for multiple comparisons.

RT = reaction time.

**Supplementary Figure B.1 Trajectories of cortical thickness for each individual subject between Visits 1 and 2**

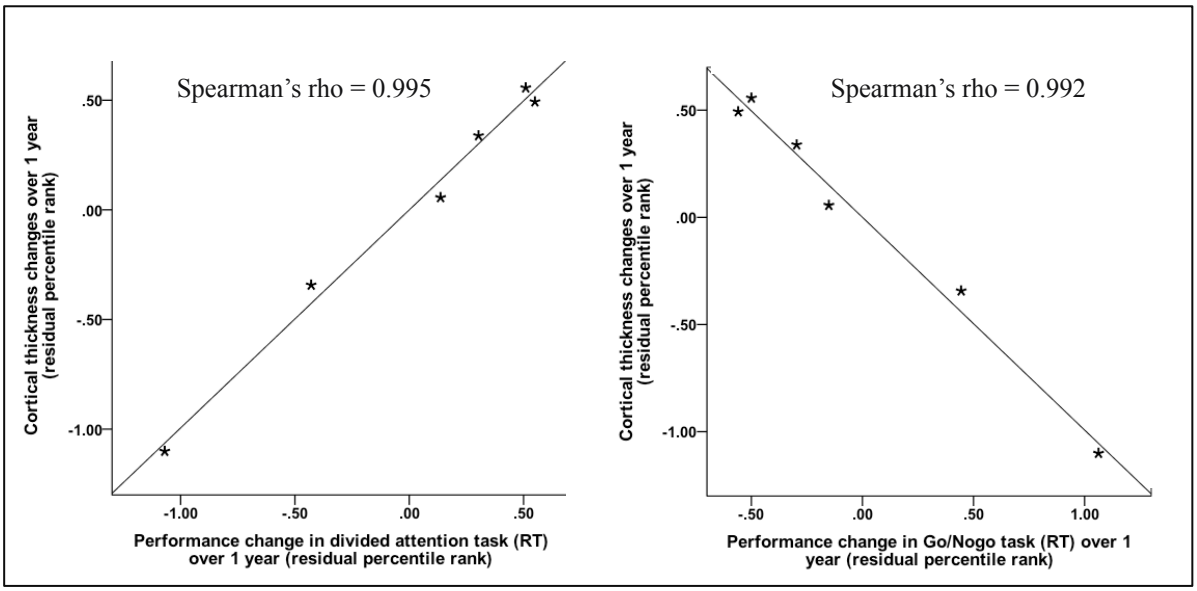


**Supplementary Figure B.2 Group difference in cortical thickness at Visit 2 using a whole brain approach**




*Note:* Group difference in cortical thickness at Visit 2 using a whole brain approach. Cortical thickness is increased (red-yellow) in bilateral anterior prefrontal cortex (APFC), left medial prefrontal cortex (MPFC), superior and middle occipital gyrus (SOG, MOG) and right intraparietal sulcus (IPS) in mTBI patients, compared with controls.

**Supplementary Figure B.3 Partial Spearman correlations between average prefrontal cortical thickening and changes in the Go/Nogo and divided attention task across time within the poor outcome subgroup of patients**



# Curriculum Vitae

## Personal data

| | |
|---|---|
| Name | Patrizia Dall'Acqua |
| Date, place of birth | 20 June 1979, Lugano (TI) |
| Nationality | Swiss |

## Education

| | |
|---|---|
| Fall 2012 – Spring 2017 | **PhD Student** at the University of Zurich, Institute of Psychology, Department of Neuropsychology<br><br>Doctoral Program Psychology (University of Zurich)<br><br>Doctoral Thesis: *Recovery-Related Brain Alterations after Mild Traumatic Brain Injury: A Longitudinal, Multimodal Imaging Approach*<br><br>Supervisors: Prof. Dr. rer. nat. Lutz Jäncke, Prof. Dr. med. Sönke Johannes<br><br>Funded by the research fund of the Swiss Accident Insurance (SUVA) |
| 11/2009 – 06/2011 | **Master of Advanced Studies in Neuropsychology** (MASNP), University of Zurich – Case presentations and supervision (Part 2)<br><br>Master thesis: *Aufmerksamkeitsaktivierung und Fatigue bei Patienten mit Multipler Sklerose: Wie hängen diese zusammen?* (Supervisor: PD Dr. P. Calabrese) |
| 10/2006 – 10/2008 | **Diploma of Advanced Studies in Neuropsychology** (DASNP), University of Zurich – Theory (Part 1) |
| 1999 – 2005 | **Master's degree of Science (M.Sc., Lic. phil.)** in Psychology; University of Zurich<br><br>Major: Neuropsychology and Cognitive Neuroscience<br><br>Master thesis: *Durch musikalisches Training bedingte Leistungsverbesserungen und damit einhergehende kortikale Aktivierungsveränderungen in motorischen Arealen in einer älteren Stichprobe* (Supervisor: Prof. Dr. Lutz Jäncke)<br>1. Minor: Child and adolescent psychopathology |

2. Minor: Communication and Media Research

1994 – 1998 Matura type B, **Gymnasium** Lugano, Canton Ticino, Switzerland

## Peer-reviewed publications

2017 **Dall'Acqua, P.**, Johannes, S., Mica, L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Jäncke, L., Hänggi, J. (2017). Prefrontal cortical thickening after mild traumatic brain injury: a one-year magnetic resonance imaging study. *J Neurotrauma,* 34, 3270–3279. doi: 10.1089/neu.2017.5124.

**Dall'Acqua, P.**, Johannes, S., Mica, L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Bätschmann, H., Jäncke, L., Hänggi, J. (2017). Functional and structural network recovery after mild traumatic brain injury: a 1-year longitudinal study. *Front Hum Neurosci,* 11 (280). doi: 10.3389/fnhum.2017.00280.

Candrian, G., Müller, A., **Dall'Acqua P.**, Kompatsiari, K., Baschera, G.-M., Mica, L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Johannes, S. (2017). Longitudinal study of a NoGo-P3 event-related potential component following mild traumatic brain injury in adults. *Ann Phys Rehabil Med*. doi: 10.1016/j.rehab.2017.07.246.

**Dall'Acqua, P.**, Müller, A., Hänggi, J., Candrian, G., Kompatsiari, K., Baschera, G.-M., Mica, L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Bätschmann, H.R., Jäncke, L., Johannes, S. (2017). Neue Erkenntnisse über die leichte traumatische Hirnverletzung - eine Langzeitstudie über 1 Jahr. *Suva Medical*, 22-36.

2016 **Dall'Acqua, P.**, Johannes, S., Mica, L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Jäncke, L., Hänggi, J. (2016). Connectomic and surface-based morphometric correlates of acute mild traumatic brain injury. *Front Hum Neurosci,* 10 (127), 1–15. doi: 10.3389/fnhum.2016.00127

2015 Müller, A., Candrian, G., **Dall'Acqua, P.**, Kompatsiari, K., Baschera, G.M., Mica, L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Johannes, S. (2015). Altered cognitive processes in the acute phase of mTBI: an analysis of independent components of event-related potentials. *Neuroreport,* 26 (16): 952-957. doi: 10.1097/WNR.0000000000000447

## Poster presentations at conferences

2017 **Dall'Acqua, P.**, Hänggi, J., Mica L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Jäncke, L., Johannes, S. Spontaneous prefrontal cortical thickening after mild traumatic brain injury: a 1-year MRI study. *1. Kooperationskongress Reha Schweiz und Physio Swiss, Davos, Switzerland, October 2017.*

2016 **Dall'Acqua, P.**, Johannes, S., Mica L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Bätschmann, H.R., Jäncke, L., Hänggi, J. Functional and structural network recovery after mild traumatic brain injury - a 1-year longitudinal study. *The 22th Annual Meeting of the Organization of the Human Brain Mapping 2016, Geneva, Switzerland, July 2016.*

**Dall'Acqua, P.**, Johannes, S., Mica L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Jäncke, L., Hänggi, J. Connectomic and surface-based morphometric correlates of acute mild traumatic brain injury. *Eleventh World Congress on Brain Injury, The Hague, Netherlands, March 2016.*

2014 **Dall'Acqua, P.**, Johannes, S., Mica L., Simmen, H.P., Glaab, R., Fandino, J., Schwendinger, M., Meier, C., Ulbrich, E.J., Müller, A., Jäncke, L., Hänggi, J. Anatomical brain correlates of mild traumatic brain injury during the acute phase - A surface-based morphometry and structural connectome analysis. *Resting state and state dependent information processing in health and disease. Monte Verità, Ascona, Switzerland, October 2014.*

## Employment history

| | |
|---|---|
| 01/2017 – now | Neuropsychologist and head of the Outpatient Day Clinic at **Bellikon Rehabilitation Clinic** (80%); |
| 04/2011 – 12/2016 | Project leader of the mild traumatic brain injury (mTBI) research study at **Bellikon Rehabilitation Clinic** (100%)<br><br>Planning and organization of the project process; Coordination of diverse internal and external cooperating partners; Neuropsychological assessment of patients after mTBI (cognitive tests performance, data interpretation, psychological diagnosis); Quantitative electroencephalography (qEEG) measurements, i.a. event-related potentials; Preprocessing, statistical analyses and interpretation of structural and functional MRI imaging data. |
| 04/2011 – 12/2011 | Neuropsychologist at **Bellikon Rehabilitation Clinic** (20%); Neurological rehabilitation (out-patients) |
| 01/2010 – 03/2011 | Neuropsychologist at **Neurocentro della Svizzera Italiana, Ospedale Ospedale Civico**, Lugano (60%); Department of Neurology (in- and out-patients) |

| | |
|---|---|
| 05/2009 – 10 /2009 | Temporary employment (100%) at the real estate company Global Property Management GPM SA (front office), Lugano (TI) |
| 08/2007 – 10/2008 | Neuropsychologist at **Zürcher Höhenklinik Wald** (ZHW, 60%) and at **Zentrum für ambulante Rehabilitation** Zurich (ZAR, 40%); neurological rehabilitation (in- and out-patients) |
| 11/2006 – 07/2007 | Neuropsychologist at **Zürcher Höhenklinik Wald** (ZHW, 100%); neurological rehabilitation (in-patients) |
| 04/2006 – 06/2006 | Internship as a neuropsychologist at **HUMAINE Klinik Zihlschlacht**; neurological rehabilitation (in-patients) |
| 02/2004 – 03/2004<br>07/2004 | Student internship at **HUMAINE Clinica Sementina**; neurological rehabilitation (in- and out-patients) |